

CHANGES IN BONE MASS AND STRENGTH PROPERTIES IN FEMALE
GYMNASTS 4 TO 10 YEARS OF AGE

by

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(Under the Direction of Richard Dana Lewis)

ABSTRACT

The influences of beginning-level gymnastics training on skeletal development were assessed in prepubertal females four to eight years of age, with essentially no organized physical activity experience prior to the onset of training. The first study was conducted using a two-year prospective quasi-experimental design to examine the influence of participation in gymnastics on bone mineral accrual (Chapter 3). One hundred and ninety six female children were recruited based on the criteria that they had not participated in organized physical activity (or had limited participation <12 weeks). Approximately 80% completed the two-year study (n=156). Sixty-five of these individuals elected to enroll in recreational gymnastics classes and were compared to 78 controls participating in nongymnastic activities or no activities. At study initiation, children who elected to begin gymnastics training were significantly shorter, lighter and leaner than those choosing to participate in the control group. Furthermore, most measures of bone mineral were lower in gymnasts vs. controls at the study onset. Over two years, gymnasts experienced a significantly ($p < 0.05$) greater rate of increase in areal bone mineral density (aBMD) of the lumbar spine (3.5%) and bone area of the radius (3.6%) compared to controls. To more rigorously control for biological and maturational differences between groups, a subgroup of prepubertal gymnasts (n=31) was individually matched to a subgroup of controls (n=31) based on prepubertal development (Tanner stage I throughout the two-year investigation), race, age, height and weight. Similar to the observations in the overall sample, the gymnasts in this group had significantly ($p < 0.05$) higher rates of lumbar spine aBMD (2.7%) and total proximal femur aBMD (1.5%) accrual compared to the matched sample of controls. Those gymnasts who advanced to a higher-competition level (n=9) were compared to those who remained at a lower-noncompetitive level (n=56) and revealed that the higher level gymnasts gained more aBMD at the lumbar spine (3.9%) and radius (3.0%) compared to low-level gymnasts. Using data from the matched subgroups (n=31 per group), the study in Chapter 4 investigated the influence of gymnastics participation on geometric strength properties determined by hip structural analysis of the proximal femur (PF). No differences in structural properties existed at baseline, and over two years, gymnasts, compared to controls, had moderately greater increases in cross-sectional area, cross-sectional moment

of inertia and section modulus at the narrow neck. These relationships depended on initial weight, where gymnasts who were heavier demonstrated the greatest strength benefits over controls. Conversely, endocortical thickness increased significantly more in the controls vs. the gymnasts. Controls also had greater increases in subperiosteal width compared to gymnasts, but this relationship depended on initial weight. At the intertrochanteric region, gymnasts had moderately greater increases in cross-sectional moment of inertia and section modulus compared to controls, whereas controls had greater increases in subperiosteal width. The interactions observed for changes in cross-sectional moment of inertia and section modulus both depended on initial weight, whereas the changes in subperiosteal width depended on initial height. Over two years, gymnasts did not differ from controls in strength variables at the shaft region of the PF. Higher-level gymnasts (n=9) showed no geometric differences over time in the PF compared to a matched group of low-level gymnasts (n=9). Findings from these studies suggest that the initial two years of recreational artistic gymnastics training in prepubertal females increased aBMD at the lumbar spine and bone area of the radius beyond those observed in controls. Furthermore, the increases in aBMD of the total proximal femur with gymnastics training observed in the matched prepubertal sample (n=31 per group) translated into moderate differences observed in the structural properties of the PF, as assessed by hip structural analysis. However, the improvements that were observed depended on initial height and weight, where those gymnasts who were taller, heavier or more developmentally mature, had the greatest structural advantage over controls. It may be possible that the positive effects of gymnastics participation on estimated bone strength in the PF will emerge as these young females advance in maturity.

INDEX WORDS: Areal bone mineral density, Body composition, Bone area, Bone geometry, Bone mineral content, Children, Dietary intake, Dual energy X-ray absorptiometry, Hip structural analysis, Pubertal maturation, Recreational artistic gymnastics

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A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial
Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2003

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DEDICATION

This Dissertation is dedicated to Mary Emma Littmann and Susan Gwendda Monkhouse, two extraordinary ladies whom my family lost in early 2003. Although you are missed with deepest sadness, my life has been permanently enriched by your presence and I am grateful for your positive and loving encouragement you continually provided. For me, you both are shining stars- although far away, you are loved, admired and always present. To their surviving husbands, Douglas and Alan, the love you shared with your wives has inspired all who have ever met you to try and aspire to such marvels of adoration, family values, happiness and companionship.

ACKNOWLEDGMENTS

I am fortunate to have had the opportunity to spend my graduate years researching and interacting with young children in the areas of physical activity and nutrition. The completion of this project would not have been possible without the vehement support and guidance of the following individuals:

To my major professor, Dr. Richard Lewis, thank you for allowing me to have challenging responsibilities and major roles both in the lab and throughout the community. I have learned so much from your talents in government policymaking, grant writing, and manuscript preparation. Thank you for inviting me to participate in a wide range of events and meetings related to osteoporosis prevention. Thank you also for your kindness toward Albert and me; you and your family have become our family away from home. To my committee members, thank you for your leadership and direction throughout my graduate study; Dr. Clifton Baile, thank you for having confidence in me as an applied scientist and for sharing your enthusiasm in my Athens-Banner Herald and UGA.com stardom! Dr. Mary Ann Johnson, thank you for your invaluable lessons in the field of nutrition, both in and outside of the classroom. I appreciate all the advice and support you have given me throughout this degree, even from Germany! Dr. Rebecca Mullis, thank you for having faith in my capabilities as a young woman and a researcher. Thank you also for the opportunity to travel with you to Houston for the ASNS workshop- you have taught me the value and importance of networking through such activities. Dr. Patrick O'Connor, thank you for including me in all of the excitement

when the new additions joined your family! Although you told me, “It’s my job”, I really do appreciate all the time you set aside to answer my statistical and research design questions.

To all the graduate and undergraduate students of the Lewis Lab who helped with early morning Saturday testing sessions, I am sincerely blessed to have worked with such supportive individuals who dedicated a great deal of their time to this project: Mandy Bartlett, Alisa Toy, Dele Sessions, Kate Silvis, Kimberly Crawford, Evi Dolce, Tanya Moss, Shilesa Chandler, Kim Eggers, Marsha Hardwick, Ruth Gildea, Nancy Aburto, Jessica Principe and Elise Kayser. To Miss Salli Lehman (woo hoo!), you will be a fabulous doctor. I miss you already. Norman Pollock, without you, I would never have consumed adequate nutrition for lunch! Thank you for always offering to help, even if it meant putting off your own work. Tonya Dalton, you brightened up the lab the first day you walked in! There are not many women who can do it all, as you are able to do- Research Coordinator, Wife, Mother, and Christian. Keep singin’ girlfriend, you are as beautiful inside as you are on the outside. Thank you for always being there for me. Katy Hardy, without your help I would have statistically significantly NOT made it through this project! I appreciate all the times you changed your plans to help me on testing days. I truly don’t know how I can repay you for all those occasions. I am excited to share an office with you next year- and I’ll do my best to keep my stories to a minimum ☺

To my dearest friends and fellow graduate students: Emilia, you have been a tremendously loving and loyal supporter. Thank you for all your help in studying for physiology, preparing for scholarships and keeping me company whenever I needed a

friend. I wish you all the success and happiness that you so rightfully deserve. How will all the coffee shops in Athens survive financially without you?! Jill Slade, my ACSM roommate and dissertation buddy, good luck in Michigan, Super-Aunt! ‘My Friend Angie’ Garcia, you are a one-of-a-kind friend, who shares with me an equal admiration for berens, walking, radio talk shows and reality TV shows! Cidney Gastaldello, thank you for your friendship throughout the past 20 years. Whenever I needed my spirits lifted, you always believed in me. Stephanie Selmer, thank you for the treats and guidebooks to pre-parenting- we’re looking forward to having the time to read them now! You and Dave will be terrific parents. I can’t wait to meet my new niece/nephew!

To my mentors and colleagues: Dr. Alissa Wilson, thank you for laying the groundwork for me to continue and complete this project. You set an excellent example and you were a tough act to follow! Dr. Chris Modlesky, thank you for always answering my questions even at obscure times of the day. You respond very well to desperation in my emails! You are no doubt an avid Fightin’ Blue Hen by now- UD is lucky to have you! Dr. Shelly Nickols-Richardson, you remain an inspiration to me even from Virginia. I have learned so much from you over the past six years. Dr. Carolyn Berdanier, Dr. Nancy Canolty, Dr. Deborah Clegg and Dr. William Flatt (Dr. Sunshine)- thank you for your guidance and support, help with scholarship applications and for brightening my day, making me believe I was feeling ‘better than ever’! Dr. Michael Mouat, thank you for all the NaCl solutions you prepared for me over the years and many thanks for your help with our new centrifuge. It is always great to see that permanent smile on your face- you are a truly wonderful person. Dr. Gary Green, thank you for being so positive and supportive of my capabilities. The Blue Day book you sent me was

a big help during comprehensive exams and the dissertation process. Thank you for your infinite advice and encouragement.

To my mother in-law and father in-law, Cecily and Kobus Laing, thank you for making those overseas trips to visit us from South Africa, and thank you also for your Sunday phone calls and weekday text messages. Although it is difficult living so far apart, it is truly wonderful that we are able to stay in touch as often as we do. I look forward to seeing you soon where we can share some of these conversations in person over a few cups of tea! Thank you for supporting me wholeheartedly throughout graduate school. Thank you to my sister in-law and brother in-law, Carine and Dirke Christowitz, and their daughters Claudia and Celia, for the wonderful e-cards and phone calls. Claudia, I am so proud of you that your English is coming along so nicely. Please forgive me that my Afrikaans is not improving at the same pace- I am hoping you can give me some lessons next time we visit!

To my entire family in Australia, especially Mervyn, Beverly, Frank, Barbara, Glenn, Alan and Eleanor, who consoled me many times when phoning to discuss the arrangements for Em and Susie. Those moments must have been terribly difficult for you all- I really wish I could have been there. Thank you to Reverend Porter Taylor who helped me through the grieving process from here. To Dubba, many thanks to you and Emsie for always making me declare 'I can do whatever I set my mind to do'. Those words will stay with me forever.

To my parents, Drs. Kay and Don Monkhouse, for their unconditional love and teaching me that a Ph.D. is just 'something you do'! To Mum, thank you for always reminding me how lucky I am to be completing this degree. You never let me forget

what an enormous achievement and a great gift this process has been... despite all my complaining! Thank you for sharing all the ups and downs of this experience. To Dad- you can relax now! Thanks for the daily phone calls on your way home from work. It was fun to catch up with our daily calamities... especially for someone who doesn't like to talk on the phone! You are the most brilliant scientist I know. Thank you for sharing your passion for scholastic and athletic achievements with me. To my sister, Courtney, thank you for your friendship and laughter. When are you coming to visit!?

To our four-legged furry children, Annie and Frodo, thank you for bringing me so much joy, company and comfort when I was working in the wee-hours of the morning... Annie at my feet, and Frodo on my lap or sometimes on the keyboard itself! The two of you never really cared what project or degree I was working toward, as long as I took breaks once in a while to pat you on the head or rub your tummies!

To my husband, Albert, who chose to marry me in spite of the reality that I hid behind the 'higher education' thing in order to avoid being in the kitchen! After four years of marriage, I still feel as if I won 'the Academy Award'. You are a spectacularly loving and supportive husband, a patient and charismatic tennis partner, and a loyal employee for me in the bone study... your ability to get children excited about giving blood is an amazing gift! You will most certainly be a terrific father too. Thank you for encouraging me to pursue this degree and allowing me to put off 'real life' for a while. I look forward to beginning this new chapter in our lives... where you can take the girls to soccer (and gymnastics, of course!). I love you.

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CHAPTER 1

INTRODUCTION

Osteoporosis is the most prevalent bone disease in the United States, accounting for over \$13 billion in healthcare costs related to fractures.^{1, 2} According to the National Institutes of Health Consensus Development Program,³ "An individual who does not reach optimal (i.e., peak) bone mass during childhood and adolescence may develop osteoporosis without the occurrence of accelerated bone loss". It has been suggested that the genetic control of bone mass is expressed early in life and is steadily maintained throughout childhood and adolescence.⁴ Childhood is thought to be a key time for skeletal responses to exercise as the heightened modeling and remodeling processes have the potential to promote favorable alterations in bone mineral and bone size and shape.⁵ Since most bone mass is accumulated by the teenage years and begins to decrease as one ages,⁶⁻⁸ it is currently assumed that if bone mass accretion can be optimized during childhood, such individuals may be less likely to experience the detrimental effects resulting from osteoporosis later in life.

Artistic gymnastics is one of the most popular youth sporting activities in the United States and is engaged in by over three million females, according to USA Gymnastics and others.¹⁰ The maneuvers performed by artistic gymnasts have been shown to generate high impact forces to the skeleton, and have been recognized for having profound effects on bone mass development.¹¹⁻¹³ The unique elements performed by gymnasts have loading characteristics that are thought to maximize the osteotrophic

response in pediatric bone. Such characteristics have recently been described by Turner:¹⁴

1) Dynamic, rather than static loading, is responsible for stimulating bone adaptation. An example of this has been demonstrated in gymnast studies^{13, 15} and jumping intervention trials,^{16, 17} where unusual movements imposed on the skeleton generated the greatest osteogenic responses compared to other activities; 2) Recent work with animal models demonstrates that short bouts, rather than continuous mechanical loading exercises, are necessary to initiate skeletal adaptation,¹⁸ and 3) The adaptation of bone is ‘error driven’, suggesting that bone cells will reach a point where forces on the skeleton that are habitual and familiar, and that occur over a long period of time, will not initiate adaptation.

Exercises that include these characteristics should maximize bone mineral accrual, as seen in studies comparing adult runners vs. gymnasts.¹⁹ Although running is a weight-bearing activity, it has been observed that bone mass measures are much lower in runners than those achieved by artistic gymnasts, likely due to differences in the peak ground reaction forces produced and the other characteristics described above.¹⁹ Comparisons of college-age²⁰ and younger elite-level artistic gymnasts¹² with nongymnast athletes or controls, demonstrate that the gymnasts have significantly higher areal bone mineral density (aBMD) values (up to 36%) at most skeletal sites.

The higher bone mass in gymnasts vs. nongymnasts is likely related to the nature of gymnastics activity, but also may be related to the starting age of the sport. One commonality existing in these studies is the fact that the majority of gymnasts started training at an early age, suggesting that the exercise exposure during youth was advantageous to the skeleton. Moreover, evidence of high aBMD values in adult former athletes who began training at an early age leads to speculation that aBMD gains may

have been sustained.^{12, 21} For example, the higher aBMD values of premenarcheal gymnasts¹¹ compared to matched nongymnast controls at the total body (4%), lumbar spine (13%), femoral neck (14%) and Ward's triangle (31%), approach differences reported between adult college-aged gymnasts²⁰ and matched controls (12%, 23%, 29% and 36% of the same sites, respectively). Ultimately, the clinical significance of such gains related to osteoporosis and fracture prevention depend upon the ability of the childhood skeleton to maintain these gains into adulthood.^{3, 22} The question of whether exercise during growth can lead to the prevention of adult osteoporotic fractures, however, is unknown.⁹

While it is evident that gymnasts who begin training early in life and advance to higher levels of competition have significantly higher bone mineral compared to nonathlete controls,^{13, 15, 20} it remains uncertain if gymnasts who excel in the sport have a genetic susceptibility to higher bone mass at the onset of training (i.e., self-selection) or if the differences in aBMD result from cumulative gains throughout the maturational stages of youth. The majority of gymnastics studies performed to date have examined gymnasts only after they had advanced to a relatively high competition level. Moreover, the durations of the studies were relatively short, lasting approximately one year, with the exception of one study that continued for three years.¹³

The present study was conducted to determine the influence of the initial years of artistic gymnastics training on prepubertal bone in children with essentially no organized physical activity experience prior to the onset of training. Investigating novice gymnasts and comparable controls was the approach taken to determine if selection bias was a key factor related to the high bone mass observed in gymnasts. There are no studies

published to date that have examined children with such limited organized sport experience prior to the onset of a physical activity intervention. The principal goals of the first study (Chapter 3) were to determine if: 1) initial bone mass differences existed in four to eight year old females who participate in gymnastics training compared to females who do not engage in gymnastics training and 2) gymnastics training for 24 months significantly impacts bone mass, growth and dietary intakes in this group. Results from this study demonstrated that children electing to enroll in gymnastics activity were significantly shorter, lighter and leaner, and had lower bone mineral values compared to those who elected to perform other (or no) activities. However, over two years, gymnasts had greater gains in lumbar spine aBMD and radius bone area compared to controls. Additionally, those gymnasts who advanced to a higher competition level had greater gains in lumbar spine and radius aBMD compared to low-level gymnasts. Intakes of dietary calcium and vitamin D were not different between the groups or over time.

While DXA is a commonly used methodology for assessment of bone mineral accrual in children, and is valid for estimating risk of osteoporotic fractures in adults,²³ it is unable to provide information on the geometric properties of bone. Knowledge of these structural characteristics of bone strength, combined with aBMD measures, may identify those at risk. The assessment of structural properties of bone in children is limited.^{12, 24, 25} In a recent cross-sectional study, using a unilateral loading model of young female tennis players aged eight to 17 years of age, Bass et al.²⁴ observed greater periosteal apposition and improved bone structure in the loaded humerus of pre-, compared to peri- or post-pubertal girls. Similarly, Petit et al.²⁶ demonstrated that a seven-month exercise program had no significant effect on changes in aBMD, cross

sectional area (CSA; an index of bone strength), subperiosteal width, average endosteal diameter or section modulus in the narrow neck, intertrochanteric or shaft regions of the proximal femur, in prepubertal girls compared to controls. However, those participants who were classified as early-pubertal responded more favorably to the intervention, by demonstrating significantly greater increases in aBMD, CSA, estimated mean cortical thickness and section modulus of the femoral narrow neck relative to controls (mean age 10 years). These findings suggest that there are changes in bone structure occurring with loading, however, these changes may be more pronounced during early puberty and limited during the prepubertal years. At present, it is unknown if this relationship between loading and the changes in bone geometric properties can be detected in females as young as four years of age.

The study presented in Chapter 4 was conducted in a prepubertal sample (i.e., Tanner stage I for breast and pubic hair development throughout two years) to determine the influences of the initial years of gymnastics training on conformational changes of proximal femur using the hip structural analysis (HSA) program.²⁷ This study attempted to answer two questions: 1) Will differences be observed in the structural properties of bone within gymnast and control groups over two years? and 2) Will gymnasts who advance to a higher competition level demonstrate the greatest improvements in strength indices of the proximal femur? Over two years, gymnasts did not differ from controls in strength variables at the shaft region of the proximal femur. At the narrow neck and intertrochanteric regions, however, gymnasts demonstrated greater increases in cross-sectional moment of inertia and section modulus compared to controls. Furthermore, gymnasts had greater increases in CSA compared to controls at the narrow neck.

However, the improvements that were observed depended on initial height and weight, where those gymnasts who were taller, heavier or more developmentally mature, had the greatest structural advantage over controls. Controls had greater increases in endosteal diameter and subperiosteal width than gymnasts at the narrow neck, and greater increases in subperiosteal width at the intertrochanteric region. The overall findings from these studies suggest that the initial two years of recreational artistic gymnastics training in prepubertal children increases aBMD at the lumbar spine and bone area of the radius beyond those observed in controls, however only modest differences are observed in the structural properties of the narrow neck and intertrochanteric regions within the proximal femur, as assessed by hip structural analysis. It may be possible that the positive effects of gymnastics participation on estimated bone strength in the proximal femur will emerge as these young females advance in maturity.

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CHAPTER 2

REVIEW OF LITERATURE

In this review, the following topics will be described, based on scientific contributions currently existing in the literature: bone biology, measurement of bone mass and strength properties, growth and maturation, endocrine function, dietary intake and physical activity. In particular, the effects of gymnastics participation on these variables will be explained, with the primary focus being on bone mineral accrual during childhood.

Biology of bone

As a tissue undergoing constant change, bone has two primary functions. First, it provides structure and mechanical support to the body, aiding in movement and providing protection to organs.¹ By weight, the properties that contribute to skeletal strength and structure include inorganic (70%) and organic (25%) matrices and water (5%). The inorganic matrix is made up of hydroxyapatite $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$, which contains calcium and phosphate crystals found in and around collagen fibers, whereas the organic matrix is made up of primarily type I collagen and noncollagenous proteins that regulate skeletal growth and remodeling. Approximately 98% of the organic matrix of bone is made up of type I collagen and noncollagenous proteins, whereas 2% is made up of the cells, osteocytes, osteoblasts and osteoclasts.² Type I collagen is the primary site for bone mineralization, which takes place between the fibers. Examples of

noncollagenous proteins include osteocalcin, integrins, growth factors, proteoglycans and glycoproteins.

Another function of bone is to provide the principal location for calcium homeostasis.² Hormones, such as PTH and calcitriol, are synthesized in response to decreased circulating levels of ionized calcium. Their role is to restore calcium levels to within normal limits in circulation and in the skeleton. When the synthesis of PTH is enhanced, osteoclasts are activated to increase the bone resorptive process, there is a consequent increase in calcium reabsorption by the kidneys, and an increase in calcitriol synthesis.² Once ionized calcium levels are normalized within the circulation, calcitriol is then responsible for stimulating the production of osteoblasts, recruited to the site(s) of newly resorbed bone.

Each growing long bone has three major components: the diaphysis (shaft), the epiphyses (ends of bone) and the epiphyseal (growth) plates. Two types of bone, trabecular (cancellous) and cortical (compact), are major elements in the structural design of these components.^{3,4} Trabecular bone is spongy in appearance, provides strength and elasticity, and is found primarily in the epiphyses, as well as in the axial skeleton. Trabecular bone comprises ~20% of the skeleton and is present in higher quantities in bones that are commonly subject to fracture in elderly individuals (e.g., at the femoral neck and lumbar spine).⁴ Since trabecular bone has a larger surface area than cortical bone, it provides an environment suitable for metabolic exchange of matrix molecules and short-term calcium homeostasis to occur.^{4,5} Cortical bone is composed of densely packed layers of mineralized collagen, providing strength, mechanical structure and rigidity to the diaphysis of long bones in the appendicular skeleton.⁶ In addition, cortical

bone is necessary for providing a temporary supply of calcium during growth.³ Although trabecular bone is the most responsive to metabolic, hormonal and mechanical changes, both trabecular and cortical bone have been shown to respond to variations in growth rate and load-bearing activity.⁴⁻⁷

The basic multicellular unit (BMU) is a defined area of bone wherein both modeling and remodeling occur.⁸ Skeletal cells include osteoblasts (fully differentiated mononuclear cells that are responsible for bone formation by synthesizing and secreting type I collagen and noncollagenous proteins), osteoclasts (multinucleated cells that are responsible for bone resorption), bone lining cells (flattened osteoblasts that are inactive), and osteocytes (mature osteoblasts located within the bone matrix).^{1,2} Bone turnover is mediated by bone lining cells that receive signals from osteocytes to either activate or inhibit the actions of osteoclasts and osteoblasts.⁸ When activated, these cells collect at a 'site of origination' and then proceed to resorb (and later replace) the bone matrix.⁹ Within the BMU, the complete cycle of remodeling strives to maintain a highly structured balance between the amount of bone formed and the amount of bone resorbed to prevent a net decrease in bone mineral. Under normal conditions (as in the healthy young adult), this cycle begins with osteoclasts forming a resorption cavity, which is subsequently filled with an equal amount of new bone by osteoblasts. Here, the BMU is balanced and bone mass is conserved.⁹ In the cases of age-related estrogen withdrawal, or unloading, bone resorption occurs at a greater rate than bone formation.¹⁰⁻¹² Throughout the remodeling process, several BMUs are in the resorption phase while others are in the formation phase. Corresponding sites where remodeling is occurring contain regions within the remodeling space where a temporary loss of bone occurs

during the resorption period. This results in under-mineralized bone located within the remodeling space. When dietary, pharmacological or physical activity interventions produce temporary changes in the amount of measurable bone mineral, without always leading to sustained changes in the quantity of bone tissue present, this is referred to as the bone-remodeling transient.¹³

Mineralization of bone increases from birth to childhood, accelerates during adolescence and peaks just after the cessation of statural growth.^{3, 14} Because of this rapid period of bone mineralization and growth, childhood is considered an optimal time to intervene (either through diet, physical activity or both) to potentially alter the mass and structural properties of bone.¹⁵ During growth and skeletal maturation, bones perpetually change in shape and size. Modeling is the process responsible for skeletal growth occurring at the periosteal surface, functioning to increase the size and alter the shape of the skeleton through the action of osteoblastic formation. Bone resorption is also taking place, however bone formation is the principal action of modeling in children.¹⁶ Bone begins its modeling process during embryonic development and continues throughout childhood and adolescence. Fetal skeletal calcification begins five to nine weeks after conception and progresses in a linear relationship with growth.^{3, 14} During early stages of maturation, the process of modeling produces an uncoupled activation state where bone formation is exceeding bone resorption. Bone is added and strengthened in the specific area(s) where increasing mechanical strain is being placed, and is removed only if it is needed in other areas of the skeleton.^{5, 12, 14, 17} In the case of bone responding to the need for calcium in the blood, modeling also provides the body with the ability to break down bone matrix in order to maintain mineral homeostasis.¹⁸

Unlike adults, children have open epiphyseal plates at the ends of the long bones. As growth occurs and the plates close, longitudinal bone growth is no longer possible.¹⁹ When modeling ceases in early adulthood,^{12, 20} the process of bone remodeling continues within the skeleton. Remodeling is the basis for mineral homeostasis and bone repair. Remodeling that occurs in the young adult years involves the coupled action of osteoblasts and osteoclasts that work together to restructure areas of the skeleton, primarily at the endosteal surface.^{5, 18} As one ages past the 5th decade, this interaction between osteoblasts and osteoclasts becomes uncoupled, where osteoblasts are not able to undergo formation at the same pace as resorptive osteoclasts.^{3, 12, 21}

Biochemical markers of bone turnover

Select biochemical markers have been proposed as sensitive indicators of bone turnover and for monitoring responses to pharmacological treatments for osteoporotic bone loss.^{22, 23} Biochemical measures have also been shown to be useful determinants of the rate of bone turnover in growing children. However, because of the high variability in children, biochemical markers are intended for use primarily in conjunction with areal bone mineral density (aBMD) or other outcome measures, and should not infer exclusively that an individual is more or less likely to have adequate bone mass. Just as growth, hormonal and environmental variables influence bone mass, similar factors have been shown to affect these biochemical indices of bone turnover. In relation to young females, both blood and urine concentrations of bone turnover markers are typically at their highest levels during periods of rapid growth, beginning their decline with the onset of menarche.^{7, 24-26} For example, prepubertal levels of biochemical markers have been shown to be as much as four to five times higher than in adults, and tend to decrease

toward adult levels in the later stages of puberty.^{23, 27, 28} The pattern of change in these measures closely approximates the pattern observed with growth velocity curves, i.e., when growth is rapid during infancy and adolescence, high concentrations of bone turnover markers are present.^{24, 28}

Serum osteocalcin and urinary pyridinium crosslinks have been shown to be both useful and reliable indicators of bone formation and resorption, respectively.^{18, 29, 30} Osteocalcin is synthesized by osteoblasts and is characterized as a profuse matrix protein of bone, showing a higher sensitivity to elevated bone turnover than other indicators.^{11, 29, 31} Having three glutamic acid residues, osteocalcin possesses a strong affinity towards hydroxyapatite.¹⁸ Serum levels of osteocalcin have been used as indicators of active skeletal mineralization in children.^{7, 26, 29, 32} Serum osteocalcin tends to be elevated in young, early pubertal females, or among those reaching their growth spurt, expressing its peak at ages 10 to 12 years.³³ Osteocalcin follows a circadian rhythm, where the highest levels are found in the morning. The synthesis of osteocalcin is dependent upon the presence of several nutrients (i.e., active metabolites of vitamin D are required for synthesis, while conversion from glutamate to gamma-carboxyglutamate relies on vitamin K).³⁴⁻³⁶

Although engaging in high intensity levels of physical activity has been shown to produce enhanced rates of formation and resorption,³⁷ few studies have examined relationships between serum osteocalcin and aBMD in healthy child gymnasts. Bass et al.³⁸ observed that serum osteocalcin was significantly lower in gymnasts vs. controls, whereas Lehtonen-Veromaa et al.³⁹ did not detect any differences between groups for levels of serum osteocalcin. In the study by Bass et al.,³⁸ gymnasts had significantly

lower intakes of energy and macronutrients than controls, which may have attributed to the lower levels of serum osteocalcin observed in the gymnasts. In young children, inter- and intra-assay CVs for osteocalcin measured by radioimmunoassay are less than 11% and 5%, respectively.^{26, 29} For children aged seven to eight years, normal values have been reported as 21.7 to 30.5 ng/ml for pre- and early-pubertal children⁴⁰ and can vary from 14.6 to 64.4 ng/ml in females four to eight years of age (classified as the 5th to 95th percentiles, respectively).⁴¹

Osteoclastic bone resorption leads to the liberation of calcium and matrix constituents into the circulation. Most biochemical assessments of bone resorption involve quantifying these collagen degradation products in the urine. Pyridinolines have been identified as specific fragments of type I collagen in urine that could provide a quantitative index of the total pyridinoline pool.⁴² The urinary crosslinks of pyridinium are thought to be among the most sensitive markers of resorption.⁴³ In bone, collagen molecules are stabilized by forming a triple helix joined by these crosslinks of hydroxylysine-pyridinolines (Pyd) and lysine-pyridinolines (Dpd). The crosslinks aid in the maintenance and stability of the collagen network structure.⁴⁴ Unlike Dpd, which is derived primarily from type I collagen, Pyd crosslinks are found in larger amounts in type I collagen, type II collagen of cartilage and in other connective tissues. Therefore, their excretion rate does not reflect bone turnover exclusively. The pyridinolines exhibit a circadian variation with a peak in the early morning and their nadir in the afternoon. However, Dpd has the least day-to-day biological variability.^{45, 46} Typically, values for bone markers are expressed in milligrams creatinine. This may cause some inconsistencies, especially during growth, because urinary creatinine is affected by

increases in lean mass and glomerular filtration.⁴⁷ This augmentation of creatinine is more rapid in younger children compared to adolescents. In addition, urinary creatinine is heavily influenced by dietary sources, such as protein intake. Using high performance liquid chromatography, the interassay CVs for Pyd and Dpd are 3.8 and 5.9% respectively.³⁰ Normal values for Pyd and Dpd range from 247 to 337 and 69 to 98 nmol/mmol creatinine in prepubertal children.²⁸

Measurement of bone mass and strength

Bone densitometry

The resistance to osteoporotic fracture is dependent upon the quantity of the bone mineral present as well as the intrinsic structural properties of bone.⁶ Areal BMD is one of the most important predictors of fracture risk⁴⁸ and is the primary outcome measure in osteoporosis assessment.⁴⁹ Dual energy X-ray absorptiometry (DXA) is considered to be one of the most effective methods for measuring bone mass and aBMD.⁵⁰ Since DXA presents as a non-invasive method, producing a low radiation exposure and a short scan time, it is accepted as an accurate and precise method for assessment of bone mass in children,⁵¹⁻⁵³ having only a minimal error (~1%) in the actual variance of the measured sites.⁵⁰ DXA measures primarily bone mineral content (BMC; g), which is referred to as a “bulk” parameter. Bone strength, on the other hand, requires additional compositional information on the bone being measured. Areal BMD takes into account both BMC and the average bone area (BA; cm²) within a given frame. The measure of aBMD provides two-dimensional information on the geometry of the bone,⁵⁰ representing an areal density (g/cm²) rather than true density (g/cm³).

Fundamentally, DXA operates by using a filter to split the X-ray beam into two distinct photon energies as they move through the body.⁵³ Researchers are able to determine how much of lean vs. fat vs. bone tissue is present by measuring how much of the beam is blocked. The Hologic QDR-1000W model operates by using an x-ray tube as the radiation source, which pulses alternately between 70 and 140 keV. This technique distinguishes bone mineral content from soft tissue and is programmed to subsequently divide this soft tissue into fat and lean masses.⁵⁴

Bone mineral measures of children assessed by DXA have provided support to the assumptions that body weight, height, body composition (i.e., body fat percent vs. lean body mass), weight-bearing activity, diet and sexual maturation, are important determinants of bone mineral accrual during childhood.^{20, 55} Because DXA measurements are influenced by the size of bones, the subject's growth characteristics must be carefully considered (especially in longitudinal studies). Reliable assessment of bone mineral changes in children requires the simultaneous evaluation of aBMD, BMC, and BA during this period of growth.²⁰

Measurement of structural properties of bone

The mechanical capabilities of bone are determined by factors such as geometry [e.g., the size, shape, cortical thickness and cross-sectional area (CSA)], intrinsic material properties (i.e., stiffness and strength) and loading conditions (i.e., type and duration of a force) at a given skeletal site.^{7, 14, 56} Areal aBMD obtained using DXA is limited in its interpretation of an individual's bone strength. Unlike some of the more recently developed technologies, DXA does not distinguish between cortical and trabecular bone.⁵⁷ Several non-invasive techniques such as quantitative ultrasonometry, quantitative

computed tomography, and magnetic resonance imaging have been developed to assess the mass, geometry and intrinsic material properties of bone. When assessing bone structure three-dimensionally, a researcher is able to determine such measures as CSA, cross-sectional moment of inertia (CSMI) and section modulus. CSA is an estimation of the strength of the entire bone, (i.e., the amount of loading a bone can endure before resulting in fracture). Therefore, the larger the CSA, the more strain it can withstand. Both CSMI and section modulus are measures of bending strength, also indicating the ability of a bone to resist fracture with mechanical loading. In conjunction with CSA, comparable increases in CSMI and section modulus should result in increases in bending strength and an overall reduction in fracture risk.

Hip structural analysis

CSA, CSMI and section modulus can be estimated from DXA images using the hip structural analysis (HSA) software program designed to generate measures of geometric properties and bone strength in regions of the femoral neck.⁵⁸ This technique has inherent limitations because the measures are estimated from an existing two-dimensional DXA image, which could be subject to placement error of the hip during scanning.⁵⁹ However, this methodology is particularly useful because it has the ability to estimate strength measures from existing scans, obtained retrospectively.

Using the HSA program, three narrow regions within the proximal femur are analyzed in 5 mm cross-sectional components of bone, and include the narrow neck, intertrochanteric and shaft regions (Figure 2.1). The narrow neck region is placed across the narrowest segment of the femoral neck, the intertrochanteric region along the bisector of the neck-shaft angle, and the shaft is placed 2 cm distal to the midpoint of the lesser

trochanter. HSA measures both the aBMD and structural geometry of each region. Key outcome variables for HSA analyses are described in Table 2.1. Average cortical thickness estimates are derived based on assumptions of cross sectional shape, illustrated in Figure 2.2. Both the narrow neck and shaft regions are considered circular, whereas

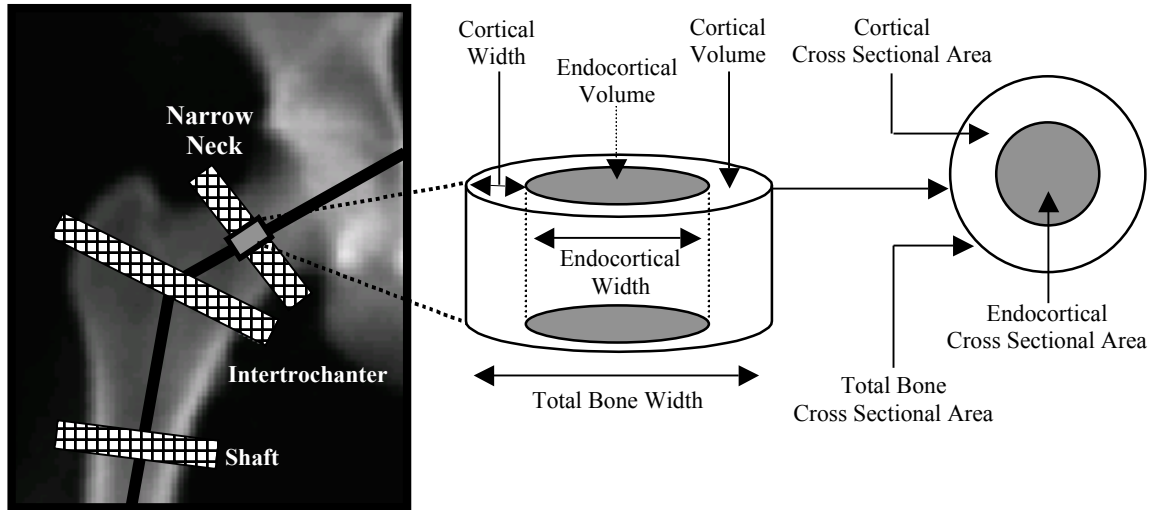


Figure 2.1. Hip structural analysis program regions of the proximal femur obtained using densitometry scan. Adapted from Modlesky and Lewis (2002)

the intertrochanteric region is assumed to be asymmetric. The intertrochanteric model assumes 70/30 proportion of cortical/trabecular bone while the narrow neck region assumes a 60/40 proportion.

Results from exercise interventions in children suggest that the proximal femur can respond to exercise by increasing bone size through either the addition of new bone to the periosteal surface, or through reduced resorption from the endocortical surface, depending on the type and force of the exercise.^{60, 61} These changes in geometric properties and improvements in strength do not necessarily result in increased aBMD using DXA. Likewise, improvements that occur in BMC and aBMD measurements do

not necessarily correlate with overall strength gains. The assessment of structural properties of bone in children is limited.^{38, 62, 63} In a recent cross-sectional study, using a unilateral loading model of young female tennis players aged eight to 17 years, Bass et al.⁶² observed greater periosteal apposition and improved bone structure in the loaded humerus of pre-, compared to peri- or post-pubertal girls.

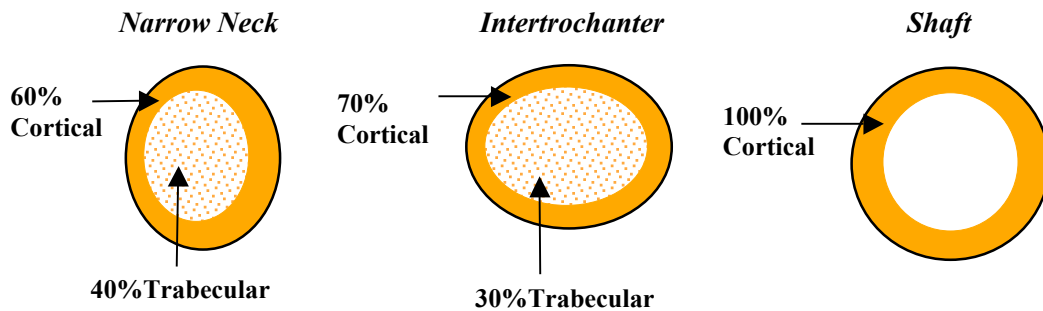


Figure 2.2. Proximal femur region models

Similarly, Petit et al.⁶⁰ demonstrated that a seven-month exercise program had no significant effect on aBMD or geometric changes in the narrow neck, intertrochanteric or shaft regions of the proximal femur, in prepubertal girls compared to controls (mean age 10 years). However, early pubertal females (Tanner stage II-III) responded more favorably to the intervention, by demonstrating significantly greater increases in aBMD, CSA, estimated mean cortical thickness and section modulus of the femoral narrow neck relative to controls. These findings suggest that there are changes in bone structure occurring with loading, however, these changes may be more pronounced during early puberty and limited during the prepubertal years. It is unknown, from the literature to date, if this relationship between loading and the changes in bone geometric properties can be detected in females of a younger age and prepubertal maturational status.

Table 2.1. Description of outcome variables produced using hip structural analysis⁵⁸

Neck length (cm)	Distance from the center of femoral head to the intersection of neck and shaft axes
aBMD (g/cm ²)	Areal Bone mineral density
CSA (cm ²)	Cross sectional area: index of axial strength; equivalent to the amount of cortical bone in the cross-section, not including the trabecular and soft tissue spaces
CSMI (cm ⁴)	Cross-sectional moment of inertia; a measure of the cross-sectional shape of the bone around the centroid used to determine the bending and torsional characteristics of bone; its value is proportional to the 4 th power of the radius
Subperiosteal width (cm)	Diameter of the bone width computed as the blur-corrected width of the mass profile
Section modulus (cm ³)	Index of bending strength; for the narrow neck and shaft regions, section modulus is taken as the [CSMI/ 1/2 subperiosteal width]; in the intertrochanteric region [CSMI/ distance from the lateral margin to the region centroid]
Endosteal diameter (cm)	Estimate of the inside diameter of the cortex
Average cortical thickness (cm)	The subperiosteal width minus [endocortical diameter / 2]
Centroid position (cm)	Distance from centroid to the medial margin / bone subperiosteal width

Childhood growth and bone

Normal pubertal development is characterized by alterations in sexual and skeletal maturation, contributing to the achievement of peak bone mass by the second decade of life.⁶⁴ The level of development of peak bone mass is principally mediated by the actions of sex steroids. In the axial skeleton, where the vertebral bodies are largely composed of

cancellous bone, the lumbar spine reaches its peak size and density following the early stages of sexual maturity. In the appendicular skeleton, which consists mostly of cortical bone, long bones continue to grow throughout adulthood by subperiosteal apposition.⁶⁵ Sites within the appendicular skeleton (e.g., the femoral neck) do not reach their peak bone mass and density until the latter stages of puberty.

The estimated heritability of bone mass accounts for 60 to 90% of its variance.^{66,}
⁶⁷ This genetic influence has been demonstrated in clinical studies where women with osteoporotic mothers had reduced bone mass compared to controls.⁶⁸ The remaining 10 to 40% of the variance in bone mass is determined by environmental factors, largely influenced by the timing of maturational development. The pre- and early-pubertal periods appear to be most adaptive to the modification of environmental conditions.²⁰ This is largely due to the varying rates of bone turnover throughout the lifespan. As illustrated in Figure 2.3, the differences in the rates of bone turnover depend largely on the maturational stage in females.

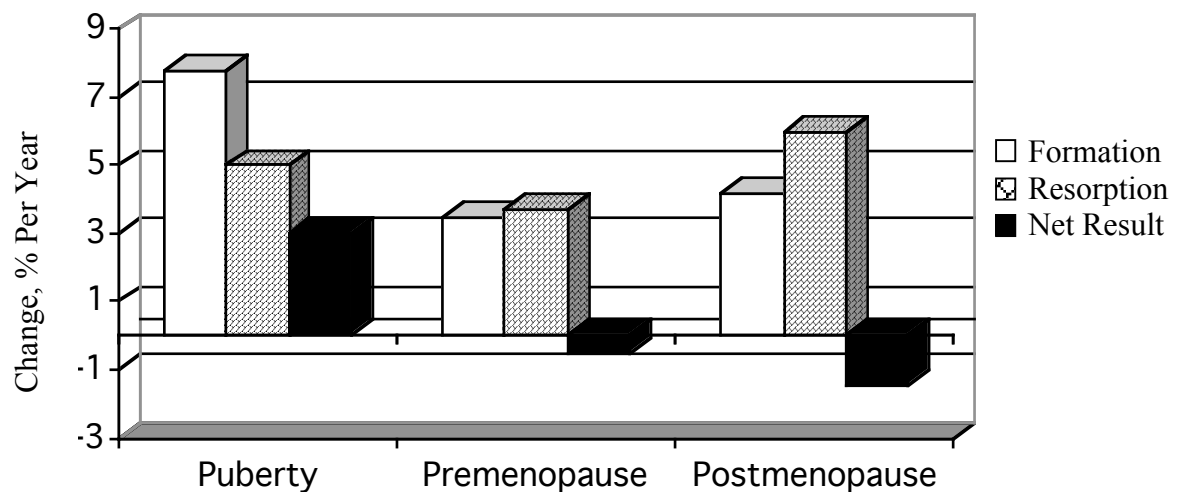


Figure 2.3. Bone Turnover Patterns in Females (Adapted from L. Riggs, Surgeon General's Workshop on Osteoporosis and Bone Health, 2002)

Growth factors

The skeleton is a reservoir for factors affecting the growth of children, such as insulin-like growth factors (IGF) and insulin-like growth factor binding proteins (IGFBP). IGFs are described as polypeptides that affect the growth and utility of skeletal cells by mediating osteoblast and osteoclast cell proliferation.^{9, 69} It has been observed that IGFs act as essential regulators of bone remodeling, working on the achievement and preservation of bone mass, likely in response to an increase in bone resorption.⁹ It is hypothesized that following osteoclastic activity, IGFs work to ensure site-specific bone formation in proportion to the bone resorbed, via systemic (e.g., PTH and calcitriol) and local (e.g., mechanical loading) stimuli.⁹

Skeletal cells synthesize a variety of growth factors, namely IGF-1 and IGF-2. IGF-1 appears to have a more potent effect than IGF-2, as it is specifically regulated by hormones affecting bone/calcium homeostasis.^{32, 34, 69-73} In children, changes in serum IGF-1 are amplified until approximately six months post menarche, indicating that the maximum IGF-1 secretion (around the time of the Tanner stage I to II transition) follows similar patterns as pubertal growth curves, markers of bone turnover and peak bone mass accrual.²⁵ Furthermore, the peak amplitude of growth hormone (GH) secretion coincides with peak height velocity, and its influence on bone is likely mediated through IGFs.⁷⁴ Growth hormone is the primary regulator of linear bone growth, whereas sex steroids, in combination with GH, act on bone during the post-pubertal years.

It has been suggested that an individual's IGF-1 status explains up to 77% of the variation in aBMD.⁷⁵ IGF-1 stimulates proliferation of osteoblast precursors and early-stage osteoblasts and promotes bone matrix formation by mature osteoblasts.^{76, 77} IGF-1

also stimulates bone resorption through enhancing recruitment, synthesis and activation of osteoclasts. In addition to the effect of IGF-1 on aBMD, there have been reports that this growth factor acts as a link between an individual's nutritional status,^{78, 79} rate of growth, muscle strength and physical activity.^{75, 80} Serum levels of IGF-1 have been shown to be positively linked to bone mineral and CSA measures in children aged seven to 18 years.⁸¹ In male gymnasts, positive and significant relationships were observed ($r = 0.67$; $p < 0.05$) between change in calcaneal ultrasound bone variables and baseline serum IGF-1.⁸² With age, there is a corresponding decline in circulating IGF-1, possibly related to both genetic and environmental factors.^{78, 79} However, acute bouts of intense exercise may activate the GH-IGF-1 axis in both pre- and early puberty. Therefore, it is possible that the best time to intervene with dietary or physical activity interventions aimed at improving bone mineral measures should be initiated when IGF-1 levels are elevated (i.e., in Tanner stages II-IV).²⁵

In circulation, IGFs are bound to specific binding proteins (also synthesized by skeletal cells).⁶⁹ IGFBPs have a high affinity and specificity for IGFs, and therefore, have the potential to influence skeletal growth and maturation.⁷⁵ One of the predominant binding proteins in circulation is IGFBP-3. IGFBP-3 serves as an important protein, as its function is to prevent both the catabolism of IGFs and any abnormal overexposure of IGFs to bone cells.^{9, 69, 75} IGFBP-3 has been shown to have a dual regulatory function with IGF-1 (either to potentiate or inhibit the effects of IGF-1, or to directly impact bone metabolism itself).⁹ Circulating levels of IGF-1 and IGFBP-3 are primarily regulated by the same factors, and although IGFBPs tend to be more stable, the complex of IGFBP-3

and IGF-1 may more actively regulate bone metabolism than the free compounds in circulation.⁷⁵

Stages of sexual and skeletal maturity

Tanner staging by physician or via self-assessment is performed by selecting an image of pubertal (breast and/or pubic hair) development from five stages.⁸³ Children in Tanner stage I are considered prepubertal, whereas Tanner stages II and III represent early puberty. Tanner stages IV and V are considered late puberty and full maturity, respectively. While this method is relatively non-invasive, especially if it is completed using self-assessment, there is a wide range of chronological ages within each Tanner stage. For example, in a study of early pubertal and prepubertal children,⁸⁴ Tanner stage II was used to classify children who were between the ages of eight and 11 years.

Skeletal growth is one of the most remarkable characteristics of puberty. During skeletal maturation, bone growth occurs and there are increases in tissue volume in order to achieve adult body size.⁸⁵ Skeletal age is considered an important predictor of bone mass, being the more precise measure of skeletal growth compared to chronological age.⁸⁶ Therefore, studies observing only chronological age may be at a disadvantage when determining bone mineral accretion. In healthy children, it has been demonstrated that up to 86% of adult bone mass of the spine is acquired before skeletal age 14 years.⁸⁷ This indicates that the adolescent response to mechanical loading may be more sensitive prior to the attainment of pubertal skeletal maturity. Elite gymnasts tend to have delayed skeletal maturity compared to nongymnast controls.⁸⁸ To date, no studies have determined if young novice gymnasts are initially shorter in stature, weigh less, or have

delayed pubertal and skeletal maturation compared to young children who do not participate in gymnastics.

Dietary intake and bone

Epidemiological studies provide evidence to suggest that nutritional habits established during childhood may lead to increased risk for adult chronic disease.⁸⁹ The nutrient composition of a child's diet has been shown to have significant effects on rates of skeletal mineralization during growth.^{20, 90} Poor rates of bone mineralization have been reported in children and adolescents who consume nutrient intakes below recommended levels, while positive effects have been observed when malnourished children are provided with nutrient supplements during growth.⁹⁰ The following discussion focuses on the dietary factors that contribute most to bone mineral accrual.

Calcium and vitamin D

It has been established that calcium, vitamin D and collagen cross linking provide the basis for bone's characteristic density and strength properties.²⁸ For this reason, calcium and vitamin D are considered the nutrients most important for attaining peak bone mass.⁹¹ Furthermore, the adequate intakes of calcium and vitamin D during childhood growth are positively correlated with bone mineralization.⁹²

Calcium exists in the form of hydroxyapatite in the bone. In the extracellular fluid (ECF), calcium is often shifted between bone and plasma in a compensatory mechanism to preserve homeostasis (i.e., to protect against hypocalcemia). This occurs mainly via parathyroid hormone (PTH) and other hormones, calcitonin and calcitriol, through the target tissues: kidneys, gastrointestinal tract and bone.^{32, 71, 93} It has been suggested that these hormones have receptors located directly on osteoblast cells, as an

acute increase in PTH can result in decreased circulating levels of osteocalcin.⁹³ In an effort to have minimal effect on tissue function, it is the calcium reserves in the bone that will be depleted when calcium intake is inadequate, or during states of high bone turnover such as menopause or skeletal development.⁷² In these extreme cases of depleted ionized calcium, there is an increase in calcium excretion combined with a decrease in both creatinine excretion and intestinal calcium absorption. These instances have been shown to inevitably lead to a degradation of bone matrix, and a decline in overall bone mass.^{72, 94} Following this withdrawal of calcium from bone, there is an increased efficiency of calcium absorption, resulting in a normalization of blood calcium levels. When there is an increase in serum calcium, there is a normalization of ECF calcium, an attenuation of PTH secretion, and a resumption of the natural rate of bone turnover.^{34, 95}

Dietary calcium intake has been shown to be an independent determinant of bone mass among children^{96, 97} and adults.⁹⁸ Studies suggest that subtle variations in calcium intake early in life may account for significant differences in peak adult bone mass.⁹⁹⁻¹⁰¹ While approaching the peak rate of skeletal volume, a vital demand for calcium occurs, where bone mineral retention may be limited by low intakes of calcium.¹⁰² In fact, consequences of failure to achieve proper dietary calcium intake have been estimated to account for a difference of as much as one standard deviation (about 10%) in bone mass accrual by the age of 18 years.¹⁰³ Dietary restriction of calcium at any age may result in an elevated rate of bone turnover⁹⁴ and, ultimately, a decrease in bone mass.¹⁰⁴ It is therefore essential that a positive calcium balance be maintained throughout various stages of the life cycle.

One of the key studies that evaluated the effects of calcium supplementation on bone mass development in children was conducted in 45 identical twin pairs.⁹⁷ The uniqueness of this study design allowed for the control of genetic influences on bone mineral accrual. One twin was given an oral calcium supplement in the amount that was twice as high as the current recommendation (1,000 mg per day of calcium citrate malate), while the control twin was given a placebo. The mean daily intake of the placebo group was 908 mg per day, while the twins receiving the calcium supplement consumed 1612 mg per day. Over three years, the twins receiving the supplement had significantly greater increases in aBMD at the radius and the lumbar spine compared to twins receiving the placebo (Figure 2.4). Because it is difficult to obtain prospective observations throughout the developmental period of childhood growth, animal models have often been used in experiments to explain the benefits of calcium on the skeleton.

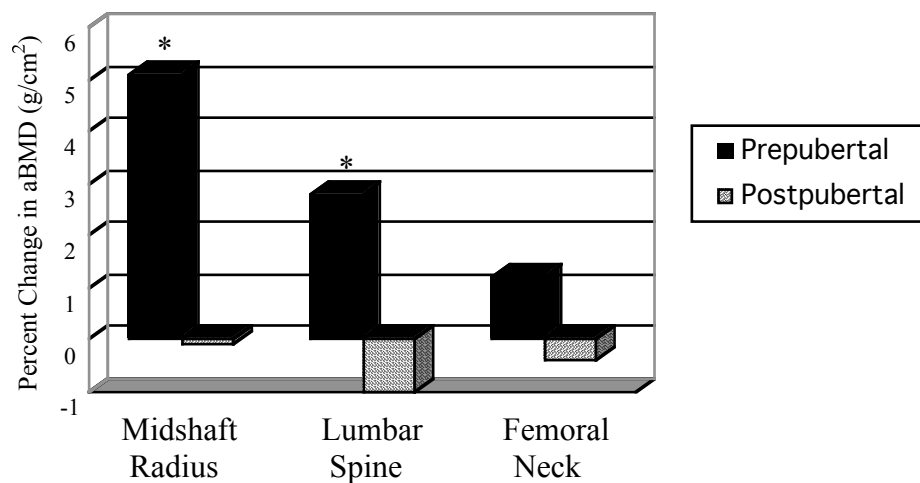


Figure 2.4. Percent difference over three years in areal bone mineral density (aBMD) between calcium-supplemented and placebo groups, according to pubertal status. *Significant difference between groups; $p < 0.05$. Adapted from Johnston et al. (1992)

In a study by Gilsanz et al.,¹⁰⁵ young rabbits were fed a diet that was either low (0.15%), normal (0.45%) or high (1.35%) in calcium from birth to skeletal maturity. The values for vertebral bone density between rabbits fed the high vs. normal calcium did not differ among groups. However, in those animals fed the low calcium diet, vertebral bone density was significantly lower throughout the period of growth. Results from this study demonstrated that decreasing dietary calcium during growth reduced peak bone mass at skeletal maturity in these animals.

In earlier calcium supplementation studies,^{33, 100, 101} it was reported that an adequate calcium intake is important in achieving peak bone mass. These studies found that the positive effects of either long-term consumption of a high calcium diet or calcium supplementation on bone mass disappeared after cessation of these practices, thereby indicating a positive role for calcium on bone acquisition. Bonjour et al.¹⁰⁰ noted that the appendicular sites appeared to be more responsive to augmented calcium than the axial skeleton. Among the appendicular regions, the most definitive effects were observed at sites containing mostly cortical bone (e.g., radial and femoral diaphysis). Studies employing diets high in calcium^{101, 106} or calcium supplementation^{100, 107} may increase bone mass by altering the bone modeling or remodeling processes. Therefore, it may be assumed that a reduction in the amount of bone turnover allows for adequate calcium uptake and mineralization.

The amount of calcium in the diet may modify the skeletal response to exercise. Two recently published randomized intervention trials have examined this issue in young children.^{108, 109} In the study by Iuliano-Burns et al.,¹⁰⁹ the effects of exercise and calcium were tested in a group of pre- and early-pubertal girls (mean age 8.8 years). The groups

were randomly assigned to receive either moderate-impact exercise with or without calcium or low-impact exercise with or without calcium. The exercise protocol consisted of 20 minutes of activity, three times per week for both groups, where the high-impact group participated in hopping, jumping, and skipping exercises, and the low-impact group performed stretching exercises and low-impact dancing routines. Participants received calcium fortified or non-fortified foods for approximately nine months. Foods were fortified with ~400 mg calcium from milk minerals and included a variety of snacks such as muffins, cookies and granola bars. The placebo group received the same foods, without the added calcium. Results from this study indicated that there was a significant exercise x calcium interaction observed at the femur ($p < 0.05$; 7.1%) assessed by DXA. Overall, BMC increased 3% more in the exercise vs. non-exercise groups ($p < 0.05$) and BMC increased 2-4% more in the calcium supplemented group vs. the placebo group at the non-loaded humerus. These results led to the conclusion that exercise generated effects at regional sites, whereas the effect of calcium was more generalized.

In the study by Specker et al.,¹⁰⁸ a similar intervention trial was conducted in children aged three to five years. Children were randomized into either ‘gross motor’ (jumping, hopping and skipping activities) or ‘fine motor’ (sedentary quiet activities) for 30 minutes a day, 5 days a week over 12 months. Within each exercise group, children either received a calcium supplement (1000 mg/day in two calcium carbonate chewable tablets) or placebo. Assessments for BMC were made by peripheral quantitative computed tomography. Results indicated that the difference between leg BMC gain between gross motor and fine motor was more pronounced in children receiving the calcium vs. placebo ($p = 0.05$ for the interaction).

Measures of sexual maturity have been viewed as chief predictors of calcium retention.¹⁰¹ A sufficient intake of dietary calcium is especially crucial during the adolescent years, when considering the rapid rate of skeletal maturation. This is because adolescents tend to retain more calcium than either children or young adults.¹⁰¹ In 1997, the National Academy of Sciences developed the Dietary Reference Intake (DRI) values, which provide the recommendations for calcium in young children [the adequate intake (AI) is 800 mg per day for children aged four to eight years].¹¹⁰ These values were derived from balance studies that estimated desirable calcium retention with varying amounts of dietary calcium. Data were then applied to a non-linear regression model. In addition, clinical trials evaluating the childhood bone response to varying levels of dietary calcium intake were considered. It is necessary for children to meet this AI for dietary calcium throughout the bone modeling period, in order for genetically predetermined peak bone mass to be achieved.¹¹¹

The role of vitamin D in bone development is critical for the effects on the active transport of calcium across intestinal mucosa, calcium absorption and calcium retention.⁷⁰ Calcitriol [1,25(OH)₂D₃] is the major biologically active metabolite of vitamin D, and the three primary target organs for calcitriol are the intestine, parathyroid gland and bone.⁷⁰⁷³ Calcitriol also plays a role in increasing the synthesis of osteocalcin, a bone matrix protein and reliable marker of bone formation. As previously discussed, the net calcium absorption and retention is highest in childhood and adolescence.⁷⁰ Consequently, calcitriol levels have also shown to be elevated with stages of rapid skeletal modeling. Ilich et al.⁷⁰ measured serum calcitriol in relation to bone mass and demonstrated that the gradual increases in aBMD, BMC and serum calcitriol concentration measures were

highest among the advanced sexual maturity stages (Tanner stages III and IV). In skeletal development, it has been suggested that the process of modeling may also result in a depletion of blood calcium levels.^{70, 73} In response to a decrease in serum calcium, circulating PTH concentrations increase rapidly, and increase renal tubular calcium reabsorption, decrease renal phosphorus reabsorption and increase calcium release from the bone.³ PTH also has a role in increasing the activity of renal tubular 1-alpha-hydroxylase, the enzyme that converts calcidiol to calcitriol, and increases active intestinal calcium transport.³

The AI for children aged four to eight years for vitamin D has been designated as 5 mcg/day. This value was based on serum 25-hydroxyvitamin D [25(OH)D] levels, and consideration for the dietary amount which has been shown to cause rickets, the clinical outcome for severe vitamin D deficiency (soft and malformed skeleton, that is unable to support body weight).¹¹⁰ Diagnosis of vitamin D deficiency in children is typically made using serum assay results of 25(OH)D less than 11 ng/ml, along with changes in linear growth and bone mass. The half-life of serum 25(OH)D is approximately two to three weeks, so it is often used as an indicator of long-term vitamin D adequacy. Although the United States has implemented policy for fortification of cow's milk with vitamin D, there are children who remain at risk for vitamin D deficiency, even in areas where sunlight exposure is not limited by geographical latitude.¹¹² Breast milk has very little vitamin D content, so infants not receiving dietary supplementation of vitamin D or adequate sunlight are at risk for developing vitamin D deficiency. In the few cases of established rickets in the state of Georgia, most cases were either due to non

supplementation of vitamin D during infancy while breastfeeding, minimal sun exposure, African-American descent, or a combination of all factors.

Other bone-related nutrients

Bone mineral deposition, maintenance and repair are cellular processes that rely on nutrition for their proper functioning. Dietary recommendations for the “bone nutrients” (calcium, phosphorus, vitamin D, magnesium and fluoride) were most recently updated in August of 1997.¹¹⁰ Recommendations for calcium and vitamin D were previously discussed. Phosphorus is needed for bone development, as it is a major component of hydroxyapatite. The RDA for phosphorus is 500 mg/day for children aged four to eight years. This value is based on estimates of need according to the factorial approach using adult serum phosphorus data. Magnesium participates in a number of biochemical reactions that take place in bone. For example, magnesium has been observed to be an essential nutrient for activating alkaline phosphatase (an enzyme involved in forming new calcium crystals), converting vitamin D to calcitriol for regulation of mineral homeostasis between the bone and serum, and for maintaining PTH secretion and the exchange of calcium and phosphorus.^{21, 113, 114} The RDA for magnesium was calculated based on achieving a positive magnesium balance in comprehensive balance studies. For females aged four to eight years, the RDA for magnesium is 130 mg/day. Fluoride mainly helps to increase bone mass at sites high in trabecular bone, by enhancing bone formation via bone cell mitogen activity.¹¹⁵ The AI for fluoride was calculated based on the prevention of dental caries. For females aged four to eight years, the AI for fluoride is 1.0 mg/day.

It has been suggested that the collagen crosslinks are the basis for providing bone with its characteristic rigidity and strength properties.²⁸ In collagen formation, vitamins K, C and D are required for its synthesis.^{113, 116} Vitamin K has a role in bone formation, as vitamin K- dependent proteins are found in osteocalcin, and bind to hydroxyapatite. Vitamin K plus vitamin D treatment has been shown to help increase aBMD in clinical cases.¹¹⁶ The AI for vitamin K for females aged four to eight is 55 mcg/day.¹¹⁷ Ascorbic acid is an essential nutrient and required cofactor in the hydroxylations of lysine and proline, which are the key factors in collagen formation.¹¹⁸ Intakes of vitamin C have been reported previously as having a positive correlation to enhanced aBMD in children.⁹⁶ If vitamin C is combined with a diet high in calcium (at least 500 mg/day) a statistically significant positive association is demonstrated between vitamin C and aBMD, suggesting that an increased production of type I collagen will result in increased bone formation, as long as sufficient calcium is present to enhance mineralization.¹¹⁸ The RDA for vitamin C for females aged four to eight years is 25 mg/day.¹¹⁹

Trace minerals such as copper, manganese and zinc have been researched to determine their essentiality as cofactors for enzymes involved in the synthesis of collagen formation.^{21, 113} The RDA for copper is 440 mcg/day for children aged four to eight years,¹¹⁷ and the AI for manganese for children aged four to eight years is 1.5 mg/day.¹¹⁷ Zinc is important in the processes related to protein synthesis, growth, food intake regulation and bone turnover. Since IGF-1 and alkaline phosphatase are zinc dependent, associations have been found between zinc deficiency and decreased levels of IGF-1, along with decreased alkaline phosphatase levels.^{113, 120} During the periods of rapid growth, the skeleton is vulnerable to dietary zinc deprivation, because heavy demands are

made on the zinc pool. In rhesus monkeys fed zinc deficient diets, Golub et al.¹²¹ observed a decrease in weight gain and linear growth during the premenarcheal growth spurt. In addition, there was slower skeletal growth, bone maturation and bone mineralization. The RDA for zinc for children four to eight years of age is 5 mg/day.¹¹⁷

Dietary intakes in child gymnasts

Adequate nutrient intake is of obvious importance for a growing child, regardless of activity level. Despite the contention that high levels of training increase the need for energy and associated nutrients, competitive gymnasts have been categorized as a population who restricts food intake in order to keep a lean physique for peak performance.¹²² Generally, there is a trend among young gymnasts to consume less than 100% of the requirement for calories, calcium and/or other selected nutrients.¹²³⁻¹²⁷ Inadequate energy intake could be dangerous to a young female, as it may contribute to menstrual irregularities¹²⁸ and/or delayed growth.¹²⁷

Several cross-sectional studies report mean intakes of energy that are below specific national recommendations,^{123, 124, 126, 129, 130} however others¹³¹⁻¹³³ report adequate intakes. With regard to energy expenditure, studies have demonstrated that gymnasts have significantly lower energy intakes compared to the amount of energy expended.¹³⁴⁻¹³⁷ Of the longitudinal studies published to date focusing on energy intake of gymnasts,^{38, 127, 138, 139} some report reduced energy intakes below recommended amounts,^{125, 127} reduced estimated needs due to energy balance¹²⁵ and decreased energy intake over time.¹²⁷

The intake of calcium during childhood growth is positively correlated with bone mineralization. Unfortunately, both cross-sectional and prospective studies reveal that

young gymnasts tend to consume below the AI for calcium,^{38, 131, 138-142} with select studies reporting calcium intakes that are less than 2/3 AI.^{38, 131, 138, 141} In a study by Benardot et al.,¹⁴³ mean calcium intakes were estimated to be above the current national recommendation, however with an intake range of 350 to 1,538 mg/day, 50% of gymnasts (aged 7-10 years) actually had intakes below those recommended.

Measurement of physical activity in children

In an early report by Sallis et al.,¹⁴⁴ it is affirmed that “The measurement of physical activity in children is an important and challenging enterprise”. Examination of fitness characteristics in a pediatric population presents unique problems and concerns with regard to developmental characteristics. Moreover, there is a need for valid, reliable and quantifiable measures of physical activity in children.¹⁴⁵ Accurate assessment of physical activity in youth is essential in studies that are designed to: 1) document the frequency and distribution of physical activity in certain population groups, 2) determine the amount of physical activity required to influence specific health outcomes, such as chronic disease risk factors, 3) identify the environmental and modifiable factors that influence physical activity behavior in children, and 4) evaluate the effectiveness of programs to increase physical activity in younger individuals.¹⁴⁶

Physical activity in a childhood population is typically measured by self-report, direct observation, heart rate monitors, doubly labeled water and/or electronic motion sensors such as accelerometers.¹⁴⁷ The instruments available to measure physical activity in children are typically chosen based on the purpose of the study, design issues, compliance of subjects, sample size and resources available to the investigator. Primarily due to concerns with expense and convenience, self-reports are most often chosen for use

in larger field studies.¹⁴⁷ Activity monitors have been developed in response to the need for reliable objective measures of activity, and to avoid the intrusiveness of both direct observation, and heart rate monitoring.¹⁴⁵ Recent advances in technology have produced sensitive activity monitors that are capable of measuring the intensity, frequency, and duration of movement for extended periods.¹⁴⁵

Questionnaires

The issues of recall errors and other biases are particularly important when administering questionnaires to children.¹⁴⁶ Most challenging is the assessment of physical activity in children under 10 years of age, as the validity of the recall depends on the cognitive development of the child.¹⁴⁸ The accuracy of the recall depends on the level of detail and format of the question, the amount of pre-training before the recall, and the use of prompting techniques. Because of age differences and children's varying cognitive ability to answer questions, there is a wide range of validity coefficients between self-report measures and more objective measures (such as direct observation and motion detection; $r = -0.10$ to 0.88).¹⁴⁶ However, like adult activities, the investigator should seek information about type, intensity, frequency and duration of each activity performed. Lastly, the investigator must decide if the accuracy of a child's physical activity recall is adequate to effectively answer the research question.¹⁴⁶

Accelerometry

Accelerometers are small electronic devices developed to measure accelerations produced by human movement.¹⁴⁶ The monitors are designed to detect motion when there is a change in the speed or pattern produced by physical activity. Accelerometers are able to detect body movement through a lever that, when displaced, generates

electrical current proportional to the energy of the acceleration. Vertical plane or unidimensional accelerometers (e.g., Caltrac and CSA; Computer Science and Applications; or more recently referred to as MTI; Manufacturing Technologies, Inc.) detect movement in a single plane, whereas multiple-plane accelerometers (e.g., Tritrac) measure movement in horizontal and vertical planes. The vertical plane monitors are able to characterize the vast majority of activities that involve walking and running, whereas the triaxial accelerometers detect vertical, horizontal and lateral movements.

Since there are limitations to the use of accelerometry, this tool cannot be considered the ‘ultimate solution’ to physical activity measurement. Many activities that are not sensitive to additional energy expenditure or are not assessed well by the device include bicycling, weight lifting, skating, stair climbing, rowing and swimming (as the devices are not waterproof). Furthermore, it is impossible to determine if a low score obtained by the monitor is due to sedentary behavior or failure to wear the instrument. Another disadvantage is that accelerometers do not record the types of physical activities that are performed. Researchers interested in this aspect of physical activity must use questionnaires or other modalities that complement accelerometry to best answer the research question.

Physical activity and bone

General levels of physical activity are positively related to the rate of bone mineral accrual during peak growth.¹⁴⁹ Weight-bearing activities that generate increased mechanical strain on the skeleton are expected to generate increases in the bone modeling process during childhood.^{150, 151} Studies in children that control for age, height and/or body weight^{142, 152} demonstrate that mean aBMD in the load-bearing group is

significantly higher than the control group for all sites measured. It was reported by Morris et al.¹⁵⁰ that aBMD and BMC of the total body, lumbar spine and femoral neck increased at a significantly greater rate than non-athletic controls in response to a high-impact, strength-building exercise intervention. Not all activities, however, produce this increase in bone mass. For instance, swimming, cycling and running have been associated with lower (or similar) bone mineral values when compared to non-athletic controls.^{140, 152-154} These studies suggest that the dynamic weight bearing forces applied to bone may be the factors responsible for initiating mineral formation. It has been proposed that for hypertrophy to occur in a specific area, the stress to this area must be greater than the load that is customary.¹⁵⁵ For instance, running is repetitive as it produces ground reaction forces of approximately two times body weight at each stride. This type of activity, therefore, does not provide the various types and intensities of strain that challenge bone to undergo mineralization, whereas gymnastics is a highly dynamic sport that exposes the bone to forces up to 10 times body weight.¹⁵⁶

Both animal⁹⁴ and human^{142, 150} studies suggest that the immature skeleton is more responsive to mechanical loading than is the adult skeleton. Childhood and adolescence are critical times for bone mineral accretion. Approximately 64% of total BMC and 86% of total aBMD are accumulated by 11 years of age.¹⁵⁷ In adults, exercise intervention studies report only slight increases (0.5 to 1.5%), no change, or a slowing of bone loss,¹⁵⁸⁻¹⁶¹ whereas children involved in youth sport activities have profoundly higher aBMD values compared to nonathletic children.^{38, 140-142, 162}

Results from studies in competitive athletes indicate that bone adapts to loading conditions to a greater extent during early puberty, rather than in adulthood. This

contention is supported by a study by Kannus et al.,¹⁶³ where BMC in the dominant and non-dominant arms of female tennis players vs. controls were examined. Bone mineral content was higher in the dominant arm in both groups, however this discrepancy was greater in the tennis players vs. controls. Furthermore, those who initiated training at an earlier age had a larger discrepancy in BMC between the dominant and non-dominant arms. Notably, the greatest BMC discrepancies were observed in a site-specific manner between those who began training before menarche and those who began training more than 15 years after menarche (Figure 2.5).

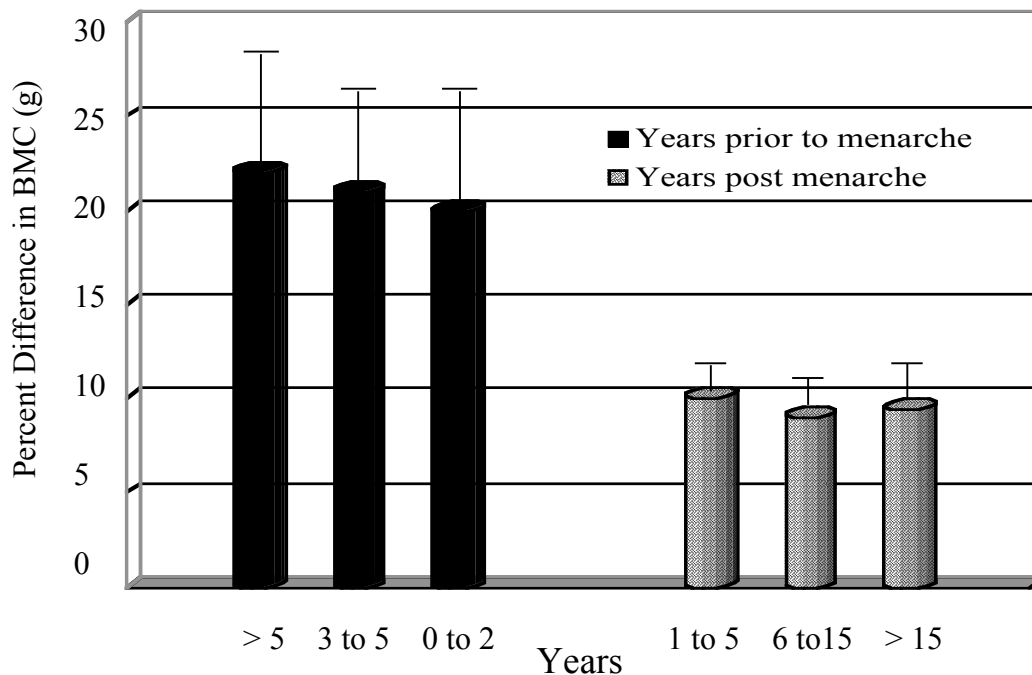


Figure 2.5. Percent difference in bone mineral content (BMC) between the dominant and non-dominant arm in female tennis players with different starting age of exercise. Error bars represent 95% Confidence Intervals. Adapted from Kannus et al. (1994)

To date, only one exercise intervention geared toward improving bone mineral accrual has been conducted in prepubertal females, who were classified as Tanner stage I throughout the experimental period.¹⁶⁴ The protocol in this study consisted of a highly-

intensive jumping program, that generated ground reaction forces of up to nine times body weight. This seven-month intervention consisted of a progressive increase from 50 to 100 drop jumps per session from a 61 cm box, three times per week for 10 minutes each time. Statistically significant improvements were found at the lumbar spine for BMC (3.1%) and aBMD (2.0%) and for BMC at the femoral neck (4.5%). Data from this investigation demonstrate that the immature skeleton is responsive to intense programs that generate a significant amount of mechanical loading, similar to what is seen by high-level gymnastics maneuvers.¹⁵⁶

High-load, weight-bearing exercises are suggested to have anabolic effects on bone tissue, and young females who are in the early stages of puberty (as opposed to prepuberty) tend to have the greatest "window of opportunity" for promoting bone mineral accrual in response to high-impact exercises.^{12, 150} Haapasalo et al.¹⁶⁵ reported side-to-side differences occurring in the humeral shaft aBMD of young tennis players and found the greatest differences taking place during the early- or peri-pubertal years (Tanner stages III and IV). In a school-based high-impact jumping intervention, Mackelvie et al.¹⁶⁶ demonstrated that early puberty vs. prepuberty was the life stage where bone was most responsive to the training. In this seven-month randomized controlled study,¹⁶⁶ children were assigned to a jumping protocol that took place outside of the regular school-based physical education classes. The exercises consisted of drop jumps and hopping and jumping over obstacles, and were implemented three times a week for 12 minutes per session. Similar to the study by Fuchs et al.,¹⁶⁴ the training program was progressive from 50 to 100 jumps per session over the seven months. Results from this study¹⁶⁶ demonstrated that the early pubertal females gained more

aBMD and BMC at the lumbar spine and femoral neck, approximately 2% more than controls at each site. However, when assessing those females who remained prepubertal, no differences were observed between exercise and control groups. One possible reason that the protocol used in the Fuchs et al.¹⁶⁴ study was able to promote significant differences at the lumbar spine and femoral neck between prepubertal groups, was likely due to the differing program intensities, where a higher level of intensity was performed compared to protocol used by Mackelvie et al.¹⁶⁶

Data generated from the Mackelvie et al.¹⁶⁶ study were analyzed in a follow up investigation to assess the structural properties of bone after a jumping intervention using HSA.⁶⁰ Results from this study were in agreement with the bone mineral accrual study, where the early pubertal children received the greatest changes in structural adaptation at the proximal femur compared to controls, whereas the prepubertal sample was not different from controls. In the early pubertal sample, the jumpers demonstrated greater changes in femoral neck bone CSA and section modulus, along with reduced endosteal expansion compared to controls.⁶⁰

Gymnastics activity and bone

Studies assessing the influence of gymnastics and bone mass in females have been relatively similar in their findings, i.e., the high-impact activities practiced by collegiate gymnasts¹⁶⁷ also appear to be beneficial for bone mineral accretion in child gymnast populations. Both cross-sectional^{131, 140-142, 168} and longitudinal^{38, 39, 139, 169} studies investigating children before puberty have demonstrated that gymnastics training is a means to promote osteogenic effects through the enhancement of peak bone mass. Cross-sectional studies by Cassell et al.,¹³¹ Courteix et al.,¹⁴⁰ Dyson et al.,¹⁴¹ Lehtonen-Veromaa

et al.¹⁶⁸ and Nickols-Richardson et al.¹⁴² have observed prepubertal female gymnasts between the ages of seven to 13 years. Results were consistent among these studies, demonstrating that gymnasts possessed significantly higher measures of aBMD at the total body (up to 9%), lumbar spine (up to 13%), total proximal femur (up to 12%), femoral neck (up to 15%), trochanter (up to 16%), Ward's triangle (up to 31%) and radius (up to 33%), respectively when compared to controls. The authors of these studies concur that physical activity in the prepubertal years is only beneficial if the type of sport induces high mechanical loading strains over an extended period of training.

Although cross-sectional studies lend support for the role of gymnastics training in enhancing aBMD in children, prospective studies are needed to determine the long-term effects of gymnastics training on bone mineral accrual. Cross-sectional studies have limitations regarding the interpretation of aBMD data, as changes cannot be assessed over time. Many cross-sectional studies have not evaluated gymnasts in comparison to controls of similar age, height and weight. Therefore, research examining homogenous groups of age, height and weight would provide more meaningful interpretations, explaining the increased aBMD experienced by gymnasts. In addition, cross-sectional studies explain current bone mass only and do not account for previous stimuli relating to bone development or sexual maturity.

Longitudinal studies are designed to examine the changes that may be occurring as gymnasts increase their hours of training, advance their level of training/competition and engage in more difficult maneuvers. The majority of prospective studies of child gymnasts published to date have reported bone mineral changes over a one-year period,^{38, 39, 139, 169} and only one followed gymnasts over three years.¹⁶² Collectively, it has been

demonstrated that prepubertal child gymnasts have greater gains in aBMD over time compared to controls.^{38, 39, 139, 169} For example, Bass et al.³⁸ examined gymnastics activity on aBMD in prepubertal elite gymnasts (mean age 10 years) and controls. Over 12 months, significant increases in total body and lumbar spine aBMD, as well as aBMD of select appendicular sites (i.e., legs and arms) occurred more rapidly in the gymnasts (30% to 85%) than the controls. In a similar study, Courteix et al.¹⁶⁹ studied highly trained prepubertal gymnasts (mean age 12 years) and non-exercising children. Over one year, gymnasts gained significantly ($p < 0.05$) more aBMD than controls at the total body (5% vs. 4%), femoral neck (5% vs. 4%), trochanter (7% vs. 5%), Ward's triangle (5% vs. 2%), and radius (21% vs. 9%).

In a study by Lehtonen-Veromaa et al.³⁹ prepubertal gymnasts (mean age 11 years) were compared to runners and nongymnast controls. After one year, gymnasts gained significantly more aBMD than controls at the lumbar spine (10% vs. 8%) and femoral neck (8% vs. 4%). In a similar study of girls aged eight to 13 years, Nickols-Richardson et al.¹³⁹ demonstrated that gymnasts had greater increases in aBMD as evidenced by the percent change from baseline to one-year at the trochanter (9% vs. 4%), femoral neck (6% vs. 4%), lumbar spine (8% vs. 7 %) and total body (6% vs. 3%).

To conclude, Laing et al.¹⁶² observed 36-month changes in aBMD among peripubertal gymnasts ($n=7$; mean age 10 years) and controls ($n=10$) of similar age, height and weight. At baseline, gymnasts possessed significantly lower percent fat and higher aBMD at all sites ($p < 0.05$), except the total body. During the three-year period, gymnasts increased up to 30% more than controls ($p < 0.05$) in total body, trochanter and total proximal femur aBMD (Figure 2.6). These results suggest that female adolescents

participating in competitive artistic gymnastics training over three-years have enhanced rates of aBMD accrual.

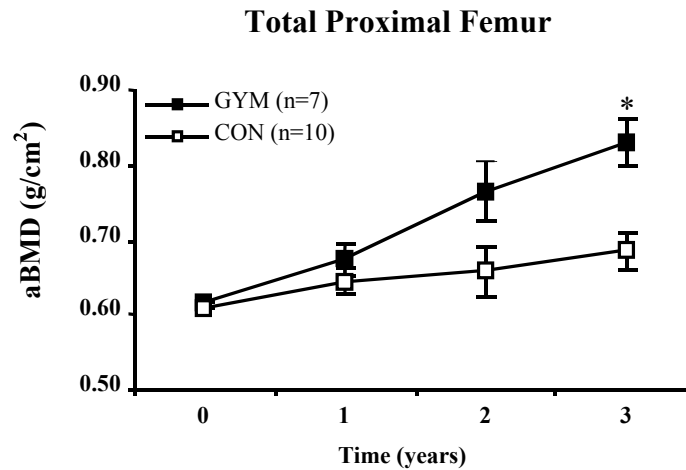


Figure 2.6. Change in total proximal femur areal bone mineral density (aBMD) over 36 months. Values are adjusted means \pm SD. *Significant ($P < 0.05$) for group differences where aBMD is higher in gymnasts than controls. Laing et al. (2002)

Results from the studies presented above suggest that strenuous athletic training can produce enhanced bone mineral accrual. The sport of gymnastics is an activity that has generated a great deal of curiosity among researchers who are interested in the mechanical aspects of bone development in children. It is consistent throughout the presented cross-sectional and prospective studies that child gymnasts have significantly higher aBMD at most measured sites compared to nongymnasts. The high-impact mechanical loading to the skeleton by the unique maneuvers performed by gymnasts during training and competition may be a major contributor.^{131, 141, 170} In fact, gymnastics routines involve ground reaction forces that place strains on the skeleton up to 10 times body weight.⁸² While the longitudinal data available for child gymnasts is promising with regard to gymnastics involvement in improving bone mineral accrual, the possibility of selection bias still remains. It is therefore plausible that individuals with

higher bone mass have an advantage in gymnastics, as they may be more likely to tolerate the rigors of high-impact training. Studies assessing bone mineral in children before the initiation of gymnastics training are needed to determine if initial differences exist between beginning-level gymnasts and nongymnast controls. To date, no studies exist that examine bone mineral in children prior to the onset of gymnastics training.

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CHAPTER 3

INITIAL YEARS OF RECREATIONAL ARTISTIC GYMNASTICS TRAINING IMPROVES LUMBAR SPINE BONE MINERAL ACCRUAL IN YOUNG FEMALES¹

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ABSTRACT

Changes in bone mineral content (BMC) and areal density (aBMD) over 24-months were examined in prepubertal females, four to eight years of age, who selected to perform beginning-level recreational gymnastics (GYM; $n = 65$), other activities, or no activity (CON; $n = 78$). Participants had essentially no history of formal athletic participation (< 12 weeks). Pubertal maturation was assessed using Tanner stages. Areal bone mineral density (aBMD; g/cm^2), bone mineral content (BMC; g) and bone area (BA; cm^2) of the total body (TB), lumbar spine (LS), total proximal femur, and radius (R) were measured at six-month intervals over 24-months using dual-energy X-ray absorptiometry (DXA; Hologic QDR-1000W). DXA Pediatric Whole Body software was used to assess fat-free soft tissue mass (FFST; g), fat mass (g), and percent body fat (%FAT). Dietary intake was estimated using 3-day diet records. Baseline differences between groups were determined using independent samples t -tests. Repeated measures analysis of covariance (RM-ANCOVA) was used to assess changes over 24-months. Covariates included initial bone response variables, height, weight and calcium intake, and changes in breast development. In order to compare the effects of advanced levels of gymnastics training on bone, gymnasts who advanced to a higher, competitive level (HLG; $n = 9$) were compared to low-level gymnasts who did not advance (LLG; $n=56$). At baseline children who were enrolled in the GYM group were shorter, lighter, leaner, and had lower BA, BMC and aBMD at all sites compared to children in the CON group ($P < 0.05$). Over two years, GYM did not differ in weight, height, sitting height or leg length compared to CON, when initial response variables and change in breast development were controlled for. Over time, GYM gained significantly more LS aBMD (3.5%; $p = 0.01$) and R BA

(3.6%; $p < 0.01$) than CON. Additional group x time interactions existed for LS BA and BMC, R BMC and TB BA, where GYM > CON ($p < 0.01$). These significant interactions were dependent, however, on initial height (LS BA, R BMC and TB BA) and bone measures (LS BMC). GYM ($n = 31$) matched to CON ($n = 31$) for prepubertal status (Tanner stage I throughout two years), race, age, height and weight, had greater gains in LS aBMD ($p < 0.01$) and TPF aBMD ($p = 0.02$). HLG increased to a greater extent than LLG in LS aBMD (3.9%; $p = 0.03$) and R BMD (3.0%; $p < 0.01$). No group x time interactions existed for fat mass, FFST or %FAT. In summary, recreational artistic gymnastics initiated at a young age promotes bone mineral gains at the LS and R over 24-months. Advancement into higher-level gymnastics participation promotes additional gains in LS and R aBMD compared to lower-level gymnastics activity.

KEY WORDS: Bone Mineral, Body Composition, Artistic Gymnastics, Pubertal Stage, Self-Selection

INTRODUCTION

Changes in the conformation of bone with exercise are dependent on the magnitude and type of loading stimulus and the timing of the loading exposure during the lifecycle.¹ Physical activities like the maneuvers performed with artistic gymnastics, produce high peak ground reaction forces and have been shown to enhance bone mineral accrual.²⁻⁴ Comparisons of college-age⁵ and younger (mean age 10 years), elite-level artistic gymnasts⁶ with nongymnast athletes or controls demonstrate that the gymnasts have aBMD values significantly higher at most skeletal sites. One commonality existing in these studies is that the majority of gymnasts started their training at an early age, suggesting that exercise exposure during youth was advantageous to the skeleton. The

immature skeleton is thought to be particularly responsive to exercise stimuli, as the heightened modeling and remodeling processes promote increases in bone mineralization and adaptations in the size and shape of bone to accommodate the loads.⁷ Laing et al.⁸ demonstrated in early pubertal competitive artistic gymnasts that three years of training led to aBMD gains almost 30% greater than nongymnasts. Results from a recent cross-sectional study by Faulkner et al.⁹ suggest that prepubescent elite-level gymnasts have greater geometric indices of strength in the proximal femur compared to controls.

While it is evident that gymnasts who begin training early in life and advance to upper levels of competition have significantly higher bone mineral compared to nonathlete controls,^{5, 8, 10} it remains uncertain if gymnasts who excel in the sport have a genetic susceptibility to higher bone mass at the onset of training or if the differences in aBMD result from cumulative gains throughout youth. The majority of gymnastics studies performed to date have examined gymnasts only after they had advanced to a relatively high competition level. Moreover, the durations of the studies were relatively short, lasting approximately one year, with the exception of the study by Laing et al.⁸

The present study was conducted to determine the influences of the initial years of artistic gymnastics training on prepubertal bone in children with essentially no organized physical activity experience *prior* to the onset of training. The use of novice gymnasts and comparable controls will help establish if selection bias is a key factor related to the high bone mass observed in gymnasts. To our knowledge, there are no studies published to date that have examined children with such limited organized sport experience prior to the onset of a physical activity intervention. Three questions were generated from this study: 1) Will there be baseline skeletal differences between prepubertal females who

elect to enroll in a recreational gymnastics program vs. those who elect to enroll in other (or no) activities?; 2) Will there be differences in the rate of bone gain between groups over two years?, and 3) Will gymnasts who advance to a higher competition level demonstrate the greatest gains in bone mineral? We hypothesized that: 1) the bone mineral characteristics of gymnasts and controls will not differ at baseline; 2) over two years, gymnasts will accrue more bone mineral and develop lean tissue at a greater rate than nongymnast controls matched for race, pubertal stage, age, height and weight, and 3) advanced, higher-level gymnasts will gain more bone mineral than recreational lower-level gymnasts.

MATERIALS AND METHODS

Design and study participants

A 24-month quasi-experimental, prospective design was used to examine the effects of recreational gymnastics activity on bone in prepubertal females (n = 203) from the Athens, Georgia, area. Females, four to eight years of age who had essentially no previous experience in organized physical activity (<12 weeks) before beginning their first gymnastics class or non-gymnastic activity, were eligible to participate in the study. All participants were apparently healthy (reporting no history of disease or conditions known to affect bone metabolism, e.g., rickets, growth hormone deficiency, use of glucocorticoid medications), and had no evidence of secondary sexual characteristics (i.e., according to Tanner staging for breast and pubic hair development) as assessed by a physician.¹¹ Twelve cohorts of children were recruited during the winter, spring, summer and fall seasons from 1997 to 2000. There were no seasonal differences at baseline between those who were recruited in the summer months vs. those recruited in the winter

months ($p > 0.49$). At baseline, participants electing to enroll in recreational gymnastics classes one hour per week (GYM) were compared to controls (CON) participating in nongymnastic activities or no activities. Of the 203 enrolled children, one hundred and ninety six children completed all baseline testing. Approximately 80% completed the two-year investigation ($n=155$), including 65 gymnasts, 78 controls and 12 participants who ceased gymnastics training and remained in the study for follow-up testing, but were not included in the statistical analyses presented here. Compared to other participants electing to enroll in gymnastics, the 12 dropouts did not differ in age, height, weight or bone mineral measures at baseline. Of the 20% who did not complete the study, 7% dropped out due to relocation, 10% due to noncompliance with the gymnastics program and the remaining 3% due to noncompliance with dietary records. The ethnic distribution of our overall sample was: 64% Caucasian, 27% African-American, 3% Asian, 2% Hispanic, 1% Indian and 3% Other (i.e., biracial).

Participants were recruited using radio and television advertisements, flyers distributed to pediatrician's offices, elementary schools and day care centers in the community, and through electronic and/or paper flyers and newsletters sent to faculty and staff members at The University of Georgia. Gymnasts were enrolled in one of three gymnastics programs in the Athens area, and trained an average of one hour per week at baseline. Introductory classes were limited to 15 students each and included a 15-minute warm-up of stretching and light activities, followed by rotations of approximately equal time on uneven bars, vault, balance beam and floor exercises. Gymnasts did not participate in other formal youth sport activities throughout the course of the investigation.

Procedures

The study protocol was approved by the Institutional Review Board for Human Subjects at The University of Georgia. Informed assent and consent were obtained from each subject and their parent, respectively. Testing procedures were completed at baseline, 6-, 12-, 18- and 24-months in the Bone and Body Composition Research Laboratory and University Health Services at The University of Georgia. On the day of testing, blood and urine samples were collected, and radiographs, sexual maturation ratings, anthropometric measures, and dual-energy x-ray absorptiometry procedures were performed. In addition, participants and their parent(s) were administered questionnaires regarding demographic information and physical activity, and were instructed on the at-home completion of 3-day diet records and use of accelerometers.

Anthropometric measures

Anthropometric measures were conducted according to the Anthropometric Standardization Reference protocol.¹² Weight of each subject wearing minimal clothing (i.e., dressed in gym shorts and top, but without shoes), was measured to the nearest 0.25 kg using a calibrated double-beam balance scale (Fairbanks Scales, Kansas City, MO). Stature was measured without shoes to the nearest 0.10 cm using a wall-mounted stadiometer (Novel Products, Inc., Rockton, IL). Sitting height was measured with each subject seated on a box 50 cm in height using the same stadiometer, and estimations for leg length were calculated using standing height minus sitting height. In our laboratory, one-way random effects model, single measure intraclass correlation (ICC) coefficients were computed for anthropometric procedures in females aged six to 10 years of age ($n = 10$). These participants were measured by the same individual twice in a two-week

period, and the ICC values were as follows: body weight ($R = 0.99$), standing height ($R = 0.99$), and sitting height ($R = 0.97$). The coefficient of variation (CV) for test-retest measures using the same children are calculated for standing height (0.4%), body weight (1.4%) and sitting height (0.9%). Stature, body weight and calculated body mass index (BMI; kg/m^2) were plotted for each child's age on growth and BMI charts¹³ to determine the percentiles for each measure.

Skeletal age assessment

Radiographs of the left hand-wrist were obtained by a qualified radiologist at baseline, 12- and 24-months for a subgroup of participants ($n=62$). Skeletal age was assessed, interpreted and analyzed by the same individual using the Fels method.¹⁴ The maturation indicators used for the Fels method have been shown to be both reliable and valid in growing children.^{14, 15} In our sample, radiographs of 12 participants ranging in age from 5.2 to 9.5 years were randomly selected and re-assessed by the same investigator approximately four months after the initial assessments were made. The mean difference between assessments of skeletal age was -0.01 ± 0.12 year, indicating a high degree of reproducibility. The ICC between assessments was $R = 0.99$, and standard errors of the estimate for skeletal age ranged from 0.26 to 0.29 years in initial and replicate assessments. The Fels method requires the measurement of diameters (to the nearest 0.5 mm) of epiphyses and metaphyses of the radius, ulna, and metacarpals and phalanges of the first, third and fifth digits. In the age range of this sample, the epiphysis of the ulna was most often not ossified. Replicate measurements were identical in 85% and within ± 0.5 mm in 15% of these assessments. In four instances, the measurements were within ± 1.0 mm, and in no instances did the replicate measurement exceed 1.0 mm.

Sexual maturation rating

Sexual maturation was conducted annually by a physician experienced in performing evaluations using a modified version of Tanner's Stages of Sexual Development rating scale (stages I to V for breast and pubic hair development).¹¹ Prepubertal is considered Tanner stage I (no evidence of breast or pubic hair development), and early pubertal is indicated by stages II to III (evidence of development).¹⁶ In our laboratory, one-way random effects model, single measure ICCs revealed perfect agreement ($r = 1.0$) for physician-assessed breast and pubic hair development in females six to 10 years of age ($n = 10$) from the current study, evaluated by the physician twice in a two-week period.

Measures of bone mineral and body composition

Areal bone mineral density (g/cm^2), bone mineral content (BMC; g) and bone area (BA; cm^2) of the total body (TB), lumbar spine (LS), non-dominant total proximal femur (TPF) and non-dominant radius (R), were determined by dual energy x-ray absorptiometry (DXA; QDR-1000W, Hologic Inc., Waltham, MA). The LS analysis was performed using DXA Low Density Spine software, whereas bone and body composition [including fat mass (g), percent fat (%FAT) and fat-free soft tissue mass (FFST; g)] measures were determined using DXA Pediatric Whole Body Analysis software. Results for the proximal femur are reported in a subgroup of $n=62$ only. Each TPF scan was analyzed by the same individual according to standard protocol described by the manufacturer. Because of the complexity of assessing TPF aBMD in young children during growth,¹⁷ the protocol for placing the region of interest (ROI) has been described. The lower border of the ROI was placed 10 spaces below the lesser trochanter (or twice

the length of the greater trochanter if not visible), five spaces outside the edge of the greater trochanter, five spaces outside the edge of the femoral head and five spaces above the edge of the femoral head. On each scan, the ischium was deleted before the densitometry values were calculated. The ROI increased with each measurement period, incrementally with growth. The femoral neck box was most often placed according to the default, but in the cases of especially young children (e.g., four years of age) where the femoral neck was not automatically recognized by the software, the left upper corner was placed in direct contact with the greater trochanter and in few instances, the width of the box was reduced to avoid inclusion of the head of the femur.

Participants wore light clothing and removed all metal items prior to conducting the scans. Quality assurance for DXA was carried out by daily calibration against the standard phantom provided by the manufacturer. A lumbar spine phantom containing calcium hydroxyapatite and epoxy sections embedded in a lucite cube (Hologic x-caliber anthropometric spine phantom, model DPA/QDR-1) was scanned each morning prior to testing. A CV of 0.27% was observed in our laboratory from 365 scans of the spine phantom over a five-year period. Quality control for soft tissue measurements was assured by concurrently scanning (with each TB scan) an external three-step soft tissue wedge composed of different thickness levels of aluminum and lucite, calibrated against stearic acid (100% fat) and water (8.6% fat; Hologic, Inc.). Test-retest measurements using DXA in young females five to eight years of age ($n = 10$) scanned twice during a one-week period demonstrated the following CVs for aBMD of the TB (1.2%), LS (1.3%), TPF (1.6%) and R (2.1%), and %FAT (2.0%). In our laboratory, one-way random effects model, single measure ICCs were calculated using the same children for

BMC and aBMD of the TB, LS, TPF and R (all $R \geq 0.98$ and 0.95 , respectively), and for %FAT (0.99).

Dietary intake measures

Dietary intake was estimated using 3-day diet records, distributed to each participant for home completion. Each parent, with assistance from their child, completed the diet record for two weekdays and one weekend day. In order to familiarize participants and parents with portion size estimations needed for completing 3-day diet records, a 24-hour recall was administered using three-dimensional plastic food models. All data were analyzed by the same individual using Food Processor Nutrition and Dietary Analysis System (Version 7.9, ESHA Research, Salem, OR). Dietary supplements of calcium and vitamin D were added subsequently to the dataset generated from the 3-day diet records. Nutrient intakes generated from the 3-day diet record have been shown to correlate highly with direct observation ($r = 0.78$ to 0.94),¹⁸ providing validity evidence for use in children. In our laboratory, one-way random effects model, average measure (i.e., 3-days) ICCs were conducted for dietary intake estimates in female children six to 10 years of age ($n = 10$), whose 3-day diet records were completed twice in a two-week period, and are calculated for energy ($R = 0.47$), calcium ($R = 0.71$) and vitamin D ($R = 0.94$).

Physical activity assessment

Physical activity was quantified objectively and subjectively using accelerometry (Model # 7164, Computer Science Applications; CSA/MTI, Fort Walton Beach, FL) and a modified version of a questionnaire developed by Slemenda et al.,¹⁹ respectively. Accelerometers were protected in a zippered pouch worn by each subject at the waist (on

the midaxillary line) for three days (two week days and one weekend day). Parents were instructed to complete a data-recording sheet indicating the periods of time when the monitor was fastened or removed from the child. Data were recorded by the CSA device during one-minute epochs. Three-day averages for activity counts per minute were generated for each subject. Janz et al.²⁰ and Trost et al.²¹ reported acceptable reliability data ($r = 0.69$ and 0.70 , respectively) from three to five-day averages in young children. The CSA monitor has also been shown to be a valid device for the assessment of children's physical activity using energy expenditure measured by calorimetry ($r = 0.87$),²² and heart rate recordings ($r = 0.50$ to 0.74).²³ Because the coaches in gymnastics and other organized sports activities discouraged the use of accelerometers during classes for safety reasons, we instructed each child in the gymnast and control groups to remove the monitor during participation in organized activities so that only leisure time physical activity would be recorded.

Gymnast and other organized control activities were quantified using the self-report questionnaire by Slemenda et al.¹⁹ Prior research with this questionnaire has provided evidence of its reliability.¹⁹ The questionnaire was interviewer-administered to each subject and their parent by trained researchers. Participants were asked to recall the time spent in after-school organized activities only. A five-point Likert scale was completed by each parent and served as an additional measure of physical activity. On this scale, parents indicated their child's usual activity level relative to childhood peers. The questionnaire consists of numerical responses indicating levels of physical activity: 1= inactive to 5= very high. Slemenda et al.¹⁹ demonstrated that parental estimates of their children's activities were positively correlated with aBMD at the hip and spine. In

addition to self-report, select gymnastics classes (i.e., low-level and high-level) were video recorded and monitored by trained observers for quantification of the type, frequency and duration of elements performed. Lastly, classes were monitored for compliance by coaches at the gymnastics facilities who submitted attendance records following each session.

Markers of bone formation and resorption

Serum osteocalcin (OC) and urinary pyridinium crosslinks are accurate and reliable indicators of bone formation and resorption, respectively. Serum intact OC was measured by radioimmunoassay.²⁴ The inter- and intra-assay CVs for OC are <10% and 5%, respectively for young individuals.²⁴ Urinary pyridinium crosslinking amino acids [pyridinoline (Pyd) and deoxypyridinoline (Dpd)] were measured by high performance liquid chromatography.²⁵ The interassay CVs for measurement of Pyd and Dpd are 3.8% and 5.9%, respectively for young healthy females.²⁵ The assessment of biochemical markers was performed on a subgroup of participants (n=62).

Statistical analyses

Data were analyzed using the Statistical Analysis System (SAS, version 8.2, SAS Institute, Cary, NC). Repeated measures analysis of covariance (RM-ANCOVA) and matched-pair RM-ANCOVA were used for statistical interpretation of the data. The resulting RM-ANCOVA models were used to model the post-baseline responses of the participants, and covariates were used to control for differences among the study groups at baseline. In addition to controlling for subject variability between groups at baseline, other covariates considered important with respect to bone changes over time were included in the model: baseline measures of the response variable, body mass, height and

calcium intake, and change in breast development.^{19, 26-28} For changes in growth variables, the initial response variable, calcium intake, and change in breast development were used as covariates. RM-ANCOVA final models were produced according to the strategy described by Milliken and Johnson.²⁹ Hypothesis 1 was addressed using independent samples *t*-tests for all baseline measures, and hypothesis 2 was addressed by testing the group x time interaction in the overall sample. In order to test hypothesis 3 that higher-level gymnasts will demonstrate greater gains in bone mineral compared to lower-level gymnasts, our overall sample of gymnasts was divided into high- vs. low-level based on the number of hours of participation in gymnastics activity. A histogram of raw hours of gymnastics classes (over the 24-month testing period) was completed and revealed a bimodal frequency distribution, where two distinct groups emerged: one lower (<100 hours; mean=63 hours over two years; average of one hour per week) and one higher activity (>100 hours; mean=259 hours over two years; average of eight hours per week; Fig. 3.1). Additionally, high-level gymnasts performed more difficult maneuvers compared to low-level gymnasts, as described in Table 3.3.

For prospective analytical comparisons, adjusted values based on RM-ANCOVA are used and statistical relationships are reported based on the independence or dependence on group interactions with other covariates. For example, ‘true’ group x time interactions are reported when results are not dependent upon other covariate interactions. Secondly, group x time interactions that depend on various levels of the covariates are reported. Effect sizes calculated for group comparisons are expressed as Cohen’s *d* (calculated as the difference between means divided by the pooled standard deviation).³⁰

For the purpose of interpreting the results, small, moderate and large effect sizes are designated as $d \geq 0.20$, 0.50 and 0.80, respectively.

Matched subgroup classifications

To more rigorously control for biological variability associated with maturation and to address hypothesis 2, a second set of analyses was performed based on a prepubertal subgroup created from our overall sample. Thirty-one children in each of the GYM and CON groups were individually matched based on the following characteristics in the order listed: sexual maturation (Tanner stage I for both breast and pubic hair through 24-months), race, age (± 1 year), height (5 cm) and weight (3.5 kg), yielding two groups of GYM_{match} and CON_{match}. Changes in this matched-pair subset were analyzed using RM-ANCOVA with effects for pair (i.e., a blocking factor) included in the model. Consistent with the statistical methods employed in the overall sample, covariates in this subgroup analysis included baseline bone response variables, calcium intake, body weight and height. Skeletal age was added as a covariate since all participants were Tanner stage I throughout the two years and both sexual and skeletal maturity can vary widely within a single Tanner stage.¹⁵ To compare the effects of high- vs. low-level gymnastics training (hypothesis 3), a second subgroup was formed based on individually matching high-level (HLG_{match}; n=9) with low-level gymnasts (LLG_{match}; n=9) employing the same matching criteria.

RESULTS

Anthropometric measures

Participant characteristics for baseline and two-year measures are presented in Table 3.1. Children electing to enroll in the GYM group were significantly shorter ($P <$

0.05; $d = 0.41$) and lighter ($P < 0.01$; $d = 0.91$) vs. those in the CON group, placing the average values for the groups (GYM vs. CON) within the 25-50th vs. the 50-75th percentiles for height and within the 50-75th vs. the 75-90th percentiles for weight, respectively.¹³ GYM had a significantly lower initial BMI ($P < 0.01$; $d = 0.52$) compared to CON, positioning the average BMI values for GYM vs. CON within the 50-75th and the 75-85th percentiles, respectively.¹³ Sitting height and leg length measures were also significantly lower in GYM ($p < 0.02$; $d > 0.20$). Over two years, GYM did not differ in weight, height, sitting height or leg length compared to CON, when initial response variables, calcium intake, and change in breast development were statistically controlled in the model.

Skeletal age and sexual maturation

By design, each participant was Tanner stage I for both breast and pubic hair development upon study entry. However, by the conclusion of the study, 11 GYM and 25 CON advanced beyond Tanner stage I, displaying some evidence of secondary sex characteristics for early pubertal breast and pubic hair development (i.e., Tanner stages II-III). While advancement in pubertal stage did occur in these individuals, menarche was not achieved during the course of the study. Skeletal age measures were assessed in our prepubertal subgroup only (i.e., 31 GYM_{match} and 31 CON_{match}) and are presented in Table 3.1. No differences existed in chronological age (5.84 ± 1.3 vs. 5.70 ± 1.3 years), skeletal age, or the difference between skeletal age and chronological age, in GYM_{match} vs. CON_{match}.

Measures of bone mineral and body composition

For each skeletal site, values are adjusted using RM-ANCOVA and statistical relationships are interpreted as: *a)* baseline group comparisons; *b)* group x time interactions over two years, not dependent on baseline covariates and *c)* group x time interactions, depending on the level of a specific covariate.

Gymnasts vs. Controls

a) Baseline bone mineral measures are presented in Table 3.1 and reveal significantly lower aBMD, BMC, and BA in GYM vs. CON at the LS, R and TB (all $P < 0.05$; $d > 0.30$). Furthermore, gymnasts had significantly lower fat mass ($P < 0.01$; $d = 0.63$), FFST ($P < 0.05$; $d = 0.42$) and %FAT ($P < 0.01$; $d = 0.46$) than controls. *b)* Compared to CON, GYM demonstrated greater gains in LS aBMD (12.6 vs. 9.1%; $P < 0.01$) and R BA (28.5 vs. 24.9%; $P < 0.01$) (Fig. 3.2). Although not significant, there was a group x time interaction for aBMD of the TB ($p = 0.09$), where GYM $>$ CON. *c)* Additional gains over time were achieved by GYM vs. CON at the LS for BA and BMC (both $p < 0.01$), however these relationships depended on interactions with height and initial BMC values, respectively. In these cases, only GYM who were taller or had higher baseline LS BMC values, demonstrated a significant gain over CON during the two-year investigation. Furthermore, GYM gained R BMC ($P < 0.01$) and TB BA ($p < 0.01$) at significantly greater rates than CON, however these relationships were also dependent on height. Significant differences were found in aBMD measures, where African-American children had higher bone mineral measures compared to Caucasian children ($p < 0.05$). When race was added as a covariate in the model to assess change over time, our overall results did not change.

Prepubertal Gymnasts vs. Controls

a) Once the groups were individually matched (i.e., GYM_{match} vs. CON_{match}) based on race, prepubertal status, chronological age, height and weight, the initial body size (i.e., height, weight, sitting height and leg length), the baseline bone and body composition differences observed between GYM and CON in the overall sample, were eliminated. *b)* Statistical comparisons of this prepubertal subgroup over time provided additional evidence that GYM_{match} gained more LS aBMD ($p < 0.01$) than CON_{match}, (10.6 vs. 7.9%). Furthermore, GYM_{match} gained TPF aBMD at a significantly greater rate than CON_{match} (10.4 vs. 8.9%; $p = 0.02$).

High-level vs. low-level gymnasts

HLG proceeded to an advanced/competition level between 6-and 12-months of beginning the sport. *b)* When comparing those with the most vs. the least hours of gymnastics participation over the two years, HLG ($n=9$) increased to a greater extent than LLG ($n=56$) in aBMD of the LS (14.9 vs. 11.0%; $p = 0.02$) and R (11.4 vs. 8.4%; $p < 0.01$; Fig. 3.3). *c)* A group x time interaction for LS BA approached significance ($p = 0.06$), however, this depended on initial age.

Prepubertal High-vs. low-level gymnasts

b) Similar in direction, but not magnitude, to the overall sample, there was a trend for prepubertal HLG_{match} to gain more bone mineral than LLG_{match} at the LS aBMD ($p = 0.10$) and R aBMD ($p = 0.09$). While not statistically significant, LLG_{match} gained more body weight ($p = 0.20$; $d = 0.69$), fat mass ($p = 0.28$; $d = 0.70$) and %FAT ($p = 0.13$; $d = 0.79$) than HLG_{match} during the study. Although HLG_{match} gained more FFST compared to LLG_{match}, the effect was small and non-significant.

Dietary intake measures

Intakes of energy, calcium and vitamin D were similar at baseline and did not change over the two years. Forty-one GYM and 35 CON reported regular use of a children's multivitamin supplement. For both GYM and CON groups, baseline mean vitamin D and calcium intakes (Table 3.1) met or exceeded the Adequate Intake recommendations for children aged four to eight years.³¹ Six GYM and five CON had individual estimated calcium intakes below 50% (400 mg/ day) of the AI, whereas 12 GYM and 11 CON had estimated vitamin D intakes below 50% (2.5 mcg/ day) of the AI.

Physical activity assessment

Baseline average activity counts per minute obtained using CSA accelerometers were not different between groups (Table 3.1). Once enrolled in the study, both GYM and CON participated in their respective youth sport activities for approximately one hour per week based on results from our activity questionnaire¹⁹ (Table 3.2). Gymnasts attended an average of 80% of scheduled classes (at least seven quarters out of a possible nine over two years) obtained from attendance records. At baseline, the average score on the parent-rated Likert scale was 3.7 ± 0.7 for GYM and 3.4 ± 0.7 for CON, indicating no significant differences between groups. Using Spearman rank-order correlations in GYM and CON, baseline parental Likert scale ratings were significantly and positively correlated with TB aBMD ($\rho = 0.25$; $p = 0.05$), and negatively correlated with fat mass ($\rho = -0.34$; $P < 0.001$) and %FAT ($\rho = -0.36$; $P < 0.001$). However, no significant differences existed for changes in this measure over time within either group.

Biochemical markers of bone turnover

Analyses of biochemical markers were completed on the prepubertal sample only (N=62). No differences existed for OC values among GYM_{match} and CON_{match} (Table 3.1). In the prospective analyses of this subgroup, OC values did not change within groups over time. For urinary measures of bone resorption, GYM_{match} had significantly lower baseline measures of Dpd than CON_{match} ($p = 0.05$; $d = 0.51$). However, no differences were observed within these groups over time.

DISCUSSION

This is the first prospective report of bone mineral changes in young children with essentially no prior structured physical activity participation. The primary finding was that over two years, young females participating in their first community-based recreational gymnastics classes gained significantly more LS aBMD, 3.5% beyond those who did not enroll in gymnastics. These results suggest that beginning-level maneuvers performed in introductory classes appear to be adequate stimuli for enhancing bone mineral accrual (as depicted in Table 3.3). We also observed that high-level, competitive gymnasts, training more than 100 hours during the course of the study (average training = eight hours per week), had greater LS aBMD and R aBMD gains, 3.9% and 3.0%, respectively, beyond the gains of gymnasts who remained at the non-competitive level (and who trained for less than 100 hours over the two years; average training = one hour per week). Compared to controls from the overall sample, high-level gymnasts gained approximately 6% more LS aBMD and R aBMD over two years.

We and others have shown that competitive collegiate,^{5, 32} adolescent^{10, 33} and retired^{6, 34} artistic gymnasts have considerably higher bone mineral measures at nearly all

skeletal sites compared to nongymnast controls similar in age, height, and weight. These higher bone values could be the result of cumulative gains with years of training, higher bone mass at the onset of the sport, or both. In more recent years, it has been documented that gymnasts initiate training as young as three to four years of age,³⁵ exposing the skeleton to years of high load stimuli. In our studies with adult competitive gymnasts, the gymnasts typically begin their training at an early age (between six⁵ and 11³⁴ years). The results from the current study support this hypothesis of cumulative gains over time. Additionally, our three-year observational study of adolescent females, (mean age 10.5 ± 1.5 years) revealed that competitive non-elite gymnasts who already had significantly higher aBMD than controls at all skeletal sites measured,¹⁰ were able to acquire additional gains in aBMD with continued training, accumulating up to 30% beyond gains in controls.⁸

Alternatively, the higher rates of bone mineral accrual in gymnasts may be the result of a genetically-inherited stronger skeleton rather than the mode of activity.³⁶ We sought to determine if there was a relationship between self-selection and bone mineral accrual in young gymnasts prior to the onset of training. Our study design allowed us to examine the anthropometric, bone and body composition measures of all participants prior to the onset of gymnastics or other organized activity involvement. Other investigations of child gymnasts were conducted at least one year after initiating the sport.^{4, 6, 37} It was therefore impossible to determine how the bone mineral measures of gymnasts compared to nongymnast controls at the start of training. In the current study, differences existed between groups at the onset, where young females who elected to participate in gymnastics were significantly shorter, lighter and leaner than those who

elected to enroll in the control group, hence, there could have been bias introduced into the study based on self-selection. Yet, the bone mineral values were lower in gymnasts, indicating that the skeletal benefits seen in this study were presumably due to the activity and not a stronger skeleton at the onset of training. To our knowledge, only one report, a retrospective study of female elite gymnasts, has shown that individuals who select gymnastics activity are smaller and leaner than controls before participation.³⁸

The 3.5% adjusted increase in LS aBMD after two years of gymnastics training corresponds with findings of shorter-duration (seven-month) childhood interventions^{2, 39} that implemented jumping programs of high-impact forces to the skeleton similar to those produced by gymnastics training. Fuchs et al.² observed a significant increase (2%) in the intervention group compared to controls for aBMD of the LS using a sample of prepubertal (Tanner stage I throughout) boys and girls, six to 10 years of age. This protocol consisted of a progressive in-school program of 10 minutes of jumping three times per week. In contrast, no differences were reported at the spine for a prepubertal sample (Tanner stage I; mean age 10.1 years) undergoing a similar jumping program.³⁹ The exercise protocol implemented in this study was 12 minutes of jumping three times per week.³⁹ Although training was progressive, the intensity of the jumping program may not have been high enough to elicit a response at the LS, as demonstrated by Fuchs et al.²

We observed that gymnasts gained significantly more LS BMC than controls. However, this relationship depended on initial BMC values, indicating that gymnasts with higher initial values had an advantage over controls in gaining BMC. These findings suggest that females who were more developmentally mature at baseline demonstrated a more pronounced skeletal response to gymnastics training. This is supported by findings

by Mackelvie et al.,³⁹ where participants classified as early-pubertal (Tanner stage II-III; mean age 10.5 years), demonstrated greater gains than prepubertal children, yielding significant increases in LS aBMD (1.7%) and BMC (1.5%).

In our study, aBMD of the LS and R increased to a greater extent in the higher-level gymnasts compared to lower-level gymnasts, up to 4%. When the prepubertal groups were individually-matched (HLG_{match} and LLG_{match}) the largest effects, although not statistically significant, were observed for LS and R aBMD in favor of the HLG_{match}. Additionally, there was a significant group x time interaction where gymnasts gained more aBMD at the TPF (1.5%) than matched controls. This finding is similar to the 1.4% increase at the TPF reported by McKay et al.,³ in pre- and early-pubertal boys and girls (mean age 8.9 years) participating in an eight-month school-based jumping intervention.

Based on direct observation (videotape analysis) of selected classes (Table 3.3), those gymnasts participating in the high-level classes performed more difficult maneuvers (e.g., jumps up and across balance beam, jump from trampoline to handstand on bar, tuck jumps on floor, and straight, tuck and straddle jumps on balance beam) than the low-level classes (e.g., forward and backward walk on balance beam, straddle and seat jumps on trampoline, and arm circles), likely generating higher ground reaction forces on the skeleton. These more advanced maneuvers would have been expected to more specifically load the TPF and promote greater aBMD gains. Zanker et al.³⁵ demonstrated that the weight bearing score in competitive child female gymnasts was eight times higher than nongymnast controls (111 vs. 14 kg²/ms x 10⁻⁵; $p < 0.01$). Likewise, Scerpella et al.⁴⁰ reported a dose- response to gymnastics training in pre-pubescent females by comparing high (>8 hrs/wk) and low (1-8 hrs/wk) level gymnasts,

where total and regional aBMD (i.e., hip and forearm) measures were augmented in the high- vs. low-level gymnasts.⁴⁰ Alternatively, an explanation for the higher TPF gains in the GYM_{match} may have been methodological. The reliability measure for the TPF conducted in our lab is 1.6%, slightly higher than the observed 1.4% difference between GYM and CON matched groups.

The development of the skeleton is characterized by distinctive biological phases and differing sequential patterns of growth in bone size and mass that are linked to hormonal regulation.^{1, 7} A principal strength of this study was the deliberate recruitment of children who were prepubertal (i.e., Tanner stage I for both breast and pubic hair development), within the age range of four to eight years of age. The purpose of employing these selection criteria was to limit variability within physiological characteristics acknowledged for their effects on the immature skeleton (e.g., pubertal maturation).^{41, 42} While groups differed at baseline concerning height, weight, sitting height and leg length, there were no group differences in these variables over two years. This was expected, as we anticipated that recreation-level artistic gymnastics initiated at a young age would not interfere with normal growth velocity, unlike the growth aberrations in elite-level gymnasts.⁶ However, longer-term studies following young females from the onset of gymnastics training throughout their growth spurt, as the intensity and duration of training intensifies, are needed to further explicate this relationship.

Within our overall sample, 17% of gymnasts and 32% of CON advanced beyond Tanner stage I over 24 months, displaying some evidence of secondary sex characteristics indicative of early puberty (no participants reached menarche). In order to minimize variation associated with biological maturation over time,¹ we were able to pair each

prepubertal gymnast to a race-, age-, height-, weight- and Tanner stage (I for both breast and pubic hair development)-matched control. Our secondary prospective analyses therefore, included only those who remained Tanner stage I throughout the duration of the study. Once this subgroup was formed, the discrepancies observed in the overall sample for anthropometric, bone and body composition values at baseline (Table 3.1) no longer existed. Importantly, the groups did not differ in baseline skeletal age or in the difference between skeletal age and chronological age.

Factors other than gymnastics training that could potentially account for differences in bone mineral accrual include dietary intakes⁴³⁻⁴⁵ and body composition.^{46,}
⁴⁷ Dietary calcium intake is an important determinant of LS aBMD among children and adolescents.^{43-45, 48, 49} Inadequate dietary calcium in children has been estimated to account for a difference of one standard deviation (~ 10%) in bone mass accrual by the age of 18 years.¹⁶ Two recently published randomized trials^{50, 51} demonstrate that over eight to 12 months, calcium intake modified the bone response to activity in young children. Iuliano-Burns et al.⁵¹ suggest that the effects of exercise are site-specific, while the effects of calcium supplementation are likely generalized. Both studies emphasize the importance of calcium in the diet with respect to bone health in young children. Dietary intakes of gymnasts and controls in the present study did not differ at baseline or over two years. The similarity between groups is consistent with prior reports in child gymnasts.^{4, 37, 52} Furthermore, both groups of participants met the Dietary Reference Intake recommendations for US children for energy,⁵³ calcium and vitamin D.³¹ These findings suggest that the higher bone gains in GYM vs CON were not influenced by the participants' calcium intakes, nor did gymnastics training alter dietary calcium intakes

over time. It is unknown, however, if intakes higher than those consumed by our participants would have potentiated the effects of gymnastics training on bone mineral accrual.

Skeletal muscle, the primary component of FFST, exerts a force on bone during muscle contraction,⁵⁴ suggesting that it is one of the most powerful determinants of aBMD acquisition (up to 60% of the variance).^{46, 47, 55} Furthermore, it has been reported that artistic gymnasts of varying ages typically have lower %FAT and/or greater FFST compared to controls.^{8, 32, 35, 56, 57} Because of these muscle-bone relationships, we anticipated that the prepubertal gymnasts in the present study would gain more FFST compared to controls. Prior to enrolling in the present study, children who elected to participate in gymnastics had significantly lower %FAT and FFST than those who elected to participate as controls. Over time, there were no differences between groups for FFST accrual. After individually-matching the groups for age and anthropometric variables, these initial body composition differences no longer existed and there were no differences observed within groups over time.

Unlike the jumping intervention studies in children,^{2, 39} we elected not to randomize children to the gymnastics intervention. While we acknowledge that causality cannot be proven in observational studies,³⁶ we gave careful consideration to the young age of the children and the likelihood that randomization into an after-school youth sport program for two years would have been unfeasible and resulted in a much higher subject attrition rate. We allowed children to self-select into either the gymnast group (beginning gymnastics classes) or the control group (participate in other sports or no sports) for the duration of the study. In an attempt to account for potential variability associated with

observational studies, we used variables such as skeletal age and other covariates important to bone mineral accrual in children that may contribute to variability associated with non-randomization.^{19, 26-28} Furthermore, the compliance with our community intervention study was 80%, similar to other exercise interventions in children (86-100%).^{2, 46}

Physical activity assessed via accelerometry was similar between groups for leisure time activity. We did not capture physical activity measured by the CSA device for participants during organized sport, and this was a limitation of this assessment. However, our data are similar to those reported by Janz et al.,⁵⁸ where the total activity counts were 701 ± 160 (expressed in average counts per minute) for nonathletic young females. While physical activity measured by accelerometry has shown to positively correlate with bone measures in preschool children,⁵⁸ this method may not be ideal for assessing bone improvements, as certain activities are not assessed well by the device (e.g., climbing and skating). In our study, parental Likert scale ratings obtained via questionnaire were significantly and positively correlated with TB aBMD and negatively correlated with %FAT and fat mass. Similarly, it has been demonstrated that children above the median of mothers' estimated activity had significantly greater measures aBMD at the hip and spine.¹⁹

Our primary outcome measure, aBMD, does not account for changes in the shape and structure of bone. Because modeling during growth can alter endosteal and periosteal dimensions,⁵⁹ measures of the structural properties of bone would have provided valuable information. Studies in children using the hip structural analysis program⁶⁰ to detect geometric and strength changes in bone have demonstrated higher indices of cross-

sectional area (an index of axial strength) and section modulus (an index of bending strength) in elite gymnasts⁹ and in children following a seven-month jumping intervention.⁵⁹

More active children may emerge from adolescence with approximately five to 10% greater bone mass depending on the skeletal site. This may signify an important biological advantage in terms of attaining optimal skeletal health and prevention of future fractures.¹⁹ It has been considered that vigorous and high loads on the skeleton are the most important exercise factors related to bone development.⁵⁸ Our data support this contention in a group of non-elite, introductory-level gymnasts of the same age. Although exercise intervention studies point to early-mid puberty as a key time for enhanced responsiveness of childhood bone, rather than during prepuberty,^{39, 59} recreational-level gymnasts in our study achieved consistently greater increases in LS aBMD compared to controls in both the overall and prepubertal samples. Furthermore, we observed that those gymnasts who advanced to a higher level of training gained more aBMD at the LS and R compared to lower-level gymnasts. There is a clear necessity for longer-term exercise intervention studies extending through the complete maturational period. A study of this nature will help contribute to our understanding of the timing of bone gains during childhood with lifestyle interventions and if a "critical period" indeed exists with respect to optimal bone mineral accrual through exercise.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the study participants and families for their enthusiasm and commitment to this project. We wish to thank Ms. Sonya Pileggi for conducting the radiographs, Dr. Robert Malina for interpreting the radiographs and

measuring skeletal age, Dr. Margaret Cramer for assessing sexual maturation, Mr. Jeff Pasley for management of the accelerometer data and Ms. Katy Hardy for assistance with the dietary analyses. In addition, we thank Mr. Edy Aguilar for conducting the blood draws, and Drs. Sue Shapses and Caren Gundberg for analyses of the biochemical data. This research was supported by National Institute on Child Health and Human Development grant 1 RO1 HD 35592-01A1. Additional financial support was provided to Emma M. Laing through the American Dietetic Association and The University of Georgia College of Family and Consumer Sciences.

TABLES AND FIGURES

TABLE 3.1. PARTICIPANT CHARACTERISTICS AT BASELINE AND TWO YEARS

	Baseline						Two Years					
	Gymnasts (n=66)			Controls (n=78)			Gymnasts (n=66)			Controls (n=78)		
<i>Age and Anthropometrics</i>												
Age (yr)	6.0	±	1.49	6.3	±	1.57	8.1	±	1.49	8.3	±	1.58
Height (cm)	115	±	10.3 [†]	119	±	11.8	128	±	10.8 [†]	133	±	11.6
Weight (kg)	21.5	±	5.10 [†]	25.2	±	7.60	28.7	±	7.57 [†]	34.8	±	11.5
BMI (kg/m ²)	16.1	±	1.67 [†]	17.3	±	2.88	17.2	±	2.51 [†]	19.2	±	4.18
Sitting Height (cm)	62.5	±	4.60 [†]	64.5	±	5.49	69.5	±	4.82 [†]	72.1	±	5.59
Leg Length (cm)	52.4	±	6.31 [†]	54.9	±	6.85	58.8	±	6.44 [†]	61.2	±	6.48
Skeletal Age (yr) ^a	6.0	±	1.21	5.9	±	1.29	8.2	±	1.30	8.0	±	1.50
<i>Dietary Intake</i>												
Energy (Kcals)	1653	±	467	1679	±	371	1749	±	364	1724	±	434
Calcium (mg)	837	±	436	847	±	337	887	±	405	805	±	323
Vitamin D (mcg)	9.4	±	6.3	8.4	±	5.5	9.5	±	6.1	8.0	±	5.8
<i>Physical Activity</i>												
Counts per minute	773	±	196	769	±	204	752	±	325	758	±	300
<i>Body Composition</i>												
Fat mass (g)	5050	±	2391 [†]	7227	±	4485	7201	±	4098 [†]	10873	±	7026
Lean mass (g)	14891	±	3228 [†]	16384	±	3873	19459	±	4670 [†]	21567	±	5302
Fat (%)	23.9	±	6.97 [†]	27.7	±	9.29	24.9	±	8.83 [†]	29.8	±	10.93
<i>Tanner Stage</i>												
Breast	1.00	±	0.00	1.00	±	0.00	1.14	±	0.39 [†]	1.32	±	0.59
Pubic Hair	1.00	±	0.00	1.00	±	0.00	1.23	±	0.55	1.24	±	0.51
<i>Total Body</i>												
BA ^b	1116	±	197 [†]	1236	±	256	1392	±	247 [†]	1545	±	314
BMC ^c	729	±	186 [†]	840	±	245	997	±	255 [†]	1151	±	323
aBMD ^d	0.646	±	0.06 [†]	0.669	±	0.06	0.708	±	0.06 [†]	0.736	±	0.07
<i>Lumbar Spine</i>												
BA	28.6	±	4.23 [†]	30.6	±	5.14	34.1	±	5.05 [†]	36.6	±	6.17
BMC	15.3	±	3.54 [†]	17.2	±	4.61	20.5	±	5.47 [†]	22.6	±	6.24
aBMD	0.530	±	0.05 [†]	0.557	±	0.07	0.592	±	0.08	0.609	±	0.08
<i>Total Proximal Femur^a</i>												
BA	13.4	±	2.72	13.3	±	2.33	17.6	±	2.86	17.4	±	2.72
BMC	7.73	±	1.99	7.90	±	2.19	11.2	±	2.23	11.2	±	2.50
aBMD	0.569	±	0.06	0.585	±	0.08	0.633	±	0.05	0.638	±	0.07
<i>Radius</i>												
BA	5.76	±	1.25 [†]	6.43	±	1.41	7.39	±	1.58 [†]	8.05	±	1.80
BMC	2.04	±	0.64 [†]	2.36	±	0.72	2.85	±	0.85 [†]	3.19	±	0.97
aBMD	0.348	±	0.04	0.361	±	0.04	0.381	±	0.04	0.391	±	0.04
<i>Biochemical Markers</i>												
OC (ng/mL) ^e	17.7	±	15.8	22.3	±	12.5	31.3	±	8.99	29.8	±	6.94
PYD (nmol/mmol Cr) ^f	175	±	49.9	154	±	54.8	162	±	56.1	170	±	52.1
DPD (nmol/mmol Cr) ^g	56.5	±	19.6 [†]	47.1	±	17.4	46.5	±	18.7	50.8	±	19.0
PYD:DPD Ratio (%)	3.22	±	0.68	3.39	±	0.81	3.67	±	0.80	3.47	±	0.69

Values are means ± SD

[†]Significant difference (p < 0.05) between Gymnasts and Controls

^aValues are for sub-sample only (Gymnasts; n=31 and Controls n=31)

^bBone area (cm²)

^cBone mineral content (g)

^dAreal bone mineral density (g/cm²)

^eOsteocalcin (Gymnasts; n=31 and Controls n=31)

^fPyridinoline (Gymnasts; n=31 and Controls n=31)

^gDeoxypyridinoline (Gymnasts; n=31 and Controls n=31)

TABLE 3.2. PARTICIPATION IN CONTROL ACTIVITIES OVER TWO YEARS

<i>Activity</i>	<i>Participation</i>	<i>Duration</i>
<i>None</i>	46	0
<i>Light/ Sedentary</i>		
Piano	1	4
Brownies/ Girl Scouts	1	2
Girls Club	6	4
Bible Camp	1	1
<i>Moderate/ Vigorous</i>		
Swimming	6	1
Tennis	2	1
Soccer	6	2
Dance	20	1.5
Karate/ Taekwondo	3	1
Twirling/ Baton	2	0.5
Horseback Riding	4	1
Basketball	8	3
Cheerleading	3	3
Softball/ Tee-Ball	4	3

Values indicate participation (n) and duration (average hours per week) in after-school activities over 24-months

TABLE 3.3. SELECTED ARTISTIC GYMNASTICS ELEMENTS FOR A) LOW-LEVEL AND B) HIGH-LEVEL CLASSES

A	Element	Number ^a
	Pike, tuck, straight and straddle jumps on long trampoline	18
	Full turn, pike, tuck, straight, and straddle jumps on floor	13
	Stick drills	3
	Hop with both feet on balance beam	3
	Jump on mini tramp to mat below	2
	Jump on springboard and bounce down to the floor	2
	Jump on springboard to higher object	2
	Cartwheels on floor	2
	Straddle jumps on circular mini-tramp	2

B	Element	Number ^b
	Jumping jacks	100
	Straddle, straight, tuck, split jumps on beam	67
	Jump to straddle handstand on floor	71
	Tuck jumps on floor	70
	Jumps up and across beam	17
	Jump from high mat rebound	15
	Pike, tuck, straight and straddle jumps on long trampoline	60
	Jump handstand over vault	4
	Jump-off end of beam backwards	1
	Jumps out and across beam	7
	Side hops over low beam	24
	Cartwheel back tuck dismount on beam	7
	Cartwheel dismount from beam onto mat	10
	Back handsprings (consecutive)	12

^aNumbers are generated for an average of 10 classes that were observed and videotaped for the low-level classes

^bNumbers are generated for an average of 5 classes that were observed and videotaped for the high-level classes

FIG. 3.1. Frequency distribution of total hours of gymnastics participation over two years. Dashed line represents designation for low-level gymnasts (LLG; <100 hours) and high-level gymnasts (HLG; >100 hours).

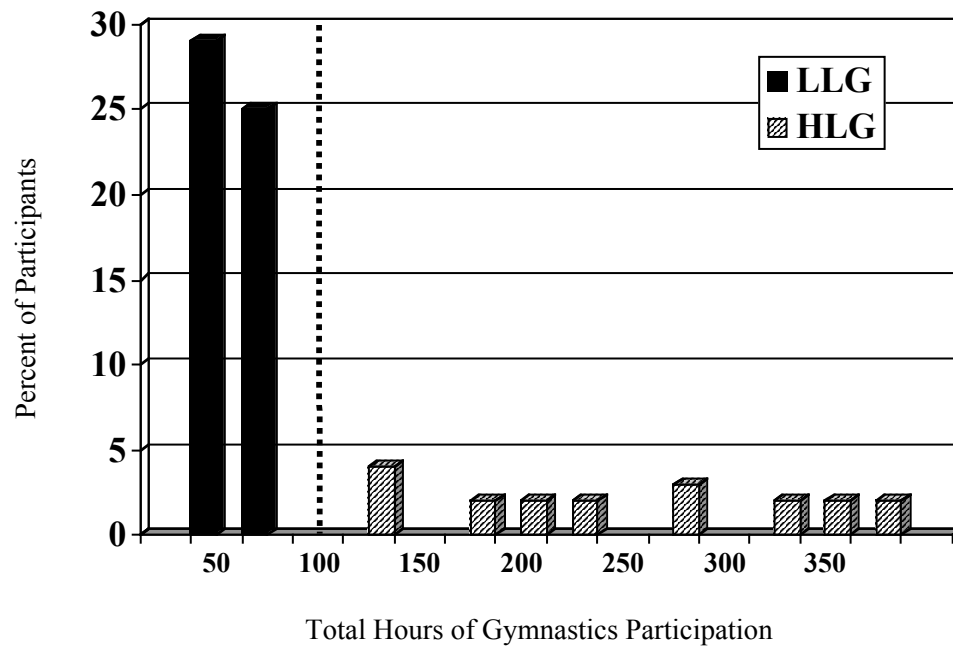


FIG. 3.2. Changes in bone area (BA; cm²), bone mineral content (BMC; g) and areal bone mineral density (aBMD; g/cm²) in the **A)** lumbar spine and **B)** radius in gymnasts (■ n=65) and controls (□ n=78) over two years. Changes in breast Tanner stage and baseline body weight, height, calcium intake and bone response variables were statistically controlled using RM-ANCOVA. [†]Group x time interaction: GYM > CON, $p < 0.01$.

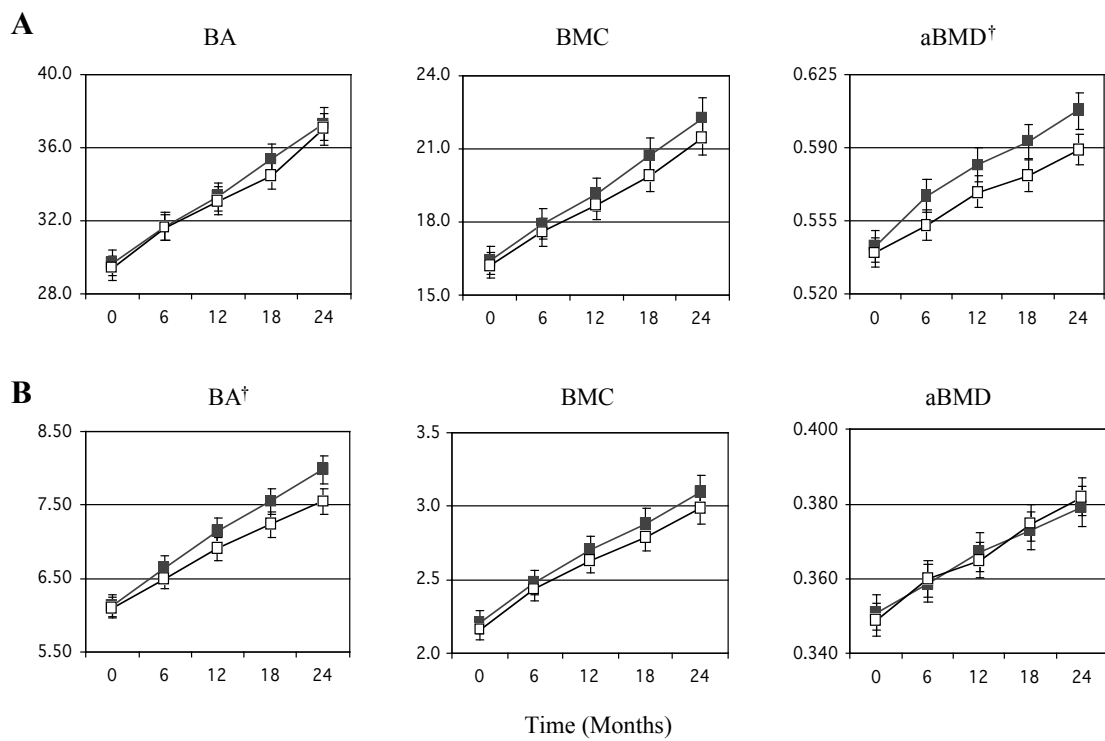
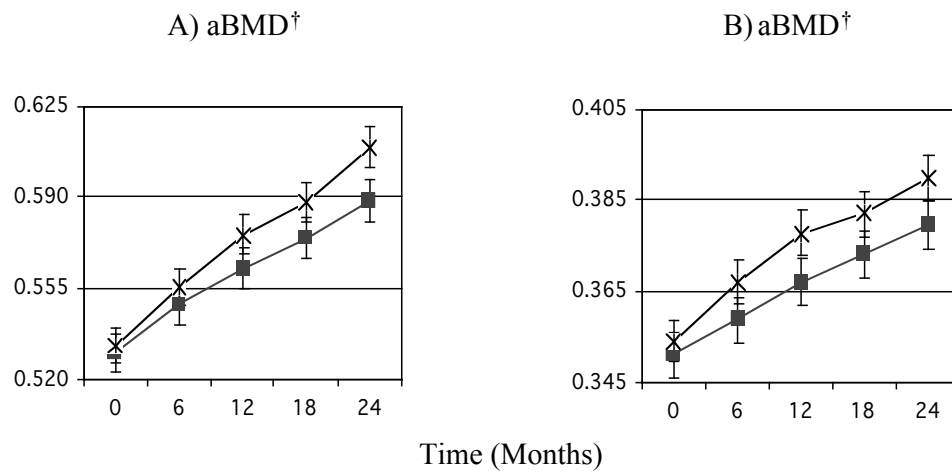


FIG. 3.3. Areal bone mineral density (aBMD; g/cm²) changes in the **A)** lumbar spine and **B)** radius of high-level gymnasts (* n=9) vs. low-level gymnasts (■ n=56) over two years; changes in breast Tanner stage as well as baseline body weight, height, calcium intake and bone response variables were statistically controlled using RM-ANCOVA.

[†]Group x time interaction: High-Level > Low-Level, $p < 0.03$.



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CHAPTER 4

STRUCTURAL PROPERTIES OF THE PROXIMAL FEMUR IN CHILD
RECREATIONAL ARTISTIC GYMNASTS¹

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ABSTRACT

Assessment of bone mineral accrual only with exercise studies is limited because important changes in the geometric properties of bone may occur and go undetected. Changes in the strength indices of the narrow neck, intertrochanteric and shaft regions of the proximal femur (PF) were examined in prepubertal female gymnasts over two years. Children who self-selected to perform recreational gymnastics (GYM; $n = 31$) were matched [by race, age, (± 1 year) height (± 5 cm) and weight (± 3.5 kg)] to a group of children who selected other activities or no activities (CON; $n = 31$). Participants remained Tanner stage I for both breast and pubic hair throughout 24 months. Prior to enrolling in the study, children had no former participation in organized athletic activities. In order to compare the effects of advanced levels of gymnastics training on structural properties of bone, gymnasts who advanced to a higher, competitive level (HLG; $n = 9$) were compared to low-level gymnasts (LLG; $n = 9$) who did not advance. Non-dominant PF scans were obtained using dual-energy X-ray absorptiometry (DXA; Hologic QDR-1000W) at baseline, 12- and 24-months. Structural properties of the PF were determined using the hip structural analysis (HSA) program, and included measurement of areal bone mineral density (aBMD) and bone strength indices [cross sectional area (CSA), cross sectional moment of inertia (CSMI), subperiosteal width, section modulus, endosteal diameter and average cortical thickness] obtained from the narrow neck, intertrochanteric and shaft regions of the PF. Baseline differences between groups were determined using independent samples *t*-tests. Repeated measures analysis of covariance (RM-ANCOVA; covariates, initial strength value, skeletal age, calcium intake, height and weight, and the ratio of fat-free mass to weight) was used to assess

changes within groups over 24-months. At baseline, the gymnast group did not differ in age, height, weight, body composition, leisure time physical activity, calcium intake or pubertal status compared to the control group. Furthermore, no initial differences were observed between groups for any of the structural properties assessed at all regions. Over two years, group x time interactions existed at the narrow neck where GYM > CON for CSA ($p = 0.06$), CSMI ($p = 0.02$) and section modulus ($p = 0.04$). Conversely, subperiosteal width ($p = 0.08$) and endosteal diameter ($p < 0.01$) increased more in CON vs. GYM. The interactions observed for changes in CSA, CSMI, section modulus and subperiosteal width all depended on initial weight. At the intertrochanteric region, group x time interactions existed where GYM > CON for CSMI ($p = 0.05$) and section modulus ($p = 0.04$), whereas subperiosteal width increased more in CON vs. GYM ($p = 0.03$). The interactions observed for changes in CSMI and section modulus both depended on initial weight, whereas the changes in subperiosteal width depended on initial height. Over two years, GYM did not differ from CON in strength variables at the shaft region of the PF. When comparing gymnasts with the most vs. the least hours of gymnastics participation over time, HLG showed no geometric differences in the PF compared to LLG. In summary, recreational artistic gymnastics initiated in prepubertal females conferred moderate geometric structural benefits at the PF. Gymnasts who were taller or heavier, demonstrated the greatest strength benefits compared to controls over time. It may be possible that the positive effects of gymnastics participation on estimated bone strength in the femur will emerge as these children advance in maturity. **Key Words:** BONE GEOMETRY, BONE MINERAL DENSITY, PREPUBERTY, RECREATIONAL ARTISTIC GYMNASTICS, HIP STRUCTURAL ANALYSIS

INTRODUCTION

Artistic gymnastics is a popular youth sport involving maneuvers that produce high peak ground reaction forces on the skeleton, up to 10 times body weight.¹ The unique elements performed by gymnasts have loading characteristics that are thought to maximize the osteogenic response in pediatric bone. Such characteristics have recently been described by Turner²: 1) Dynamic, rather than static loading, is responsible for stimulating bone adaptation. This has been demonstrated in gymnast studies^{3,4} and jumping intervention trials^{5,6} where unusual movements imposed on the skeleton generated the greatest osteogenic responses compared to other activities; 2) Recent work with animal models has demonstrated that short bouts, rather than continuous mechanical loading exercises, are necessary to initiate skeletal adaptation⁷, and 3) The adaptation of bone is ‘error driven’, suggesting that bone cells will reach a point where forces on the skeleton that are habitual, familiar and occur over a long period of time, will not initiate adaptation. Gymnastics maneuvers incorporate many of these characteristics, and should maximize bone mineral accrual as seen in studies comparing adult runners vs. gymnasts.⁸ Although running is a weight-bearing activity, it has been shown that bone mass measures are much lower in runners than those achieved by artistic gymnasts, likely due to differences in the peak ground reaction forces produced and the other characteristics described above.⁸ Comparisons of college-age⁹ and younger, elite-level artistic gymnasts¹⁰ with nongymnast athletes or controls, demonstrate that the gymnasts have significantly higher areal bone mineral density (aBMD) values (up to 36%) at most skeletal sites.

Typically, gymnasts begin recreation-level participation at an early age,^{3, 11} where the high impact forces generated by the sport are expected to have the greatest influence on bone mineral accretion in the immature skeleton, as compared to the mature adult skeleton.¹² Prospective studies have demonstrated that child gymnasts who advance to elite and non-elite levels of competition have significantly higher site-specific bone mineral accrual, measured by dual energy X-ray absorptiometry (DXA), than nonathlete controls.^{4, 10, 13}

While DXA is a commonly used methodology for assessment of bone mineral accrual in children, and is valid for estimating risk of osteoporotic fractures in adults,¹⁴ it is unable to provide information on the geometric properties of bone. Knowledge of these structural characteristics of bone strength, combined with aBMD measures, may improve the ability of identifying those at risk.¹⁹ The assessment of structural properties of bone in studies involving children is limited to cross-sectional studies of child athletes^{10, 15, 16} and exercise interventions.^{17, 18} Change in bone structure occurs with loading, however the changes may be more pronounced during early puberty, and limited during the prepubertal years. In a recent cross-sectional study, using a unilateral loading model of pre, peri- and post-pubertal female tennis players, Bass et al.¹⁵ observed greater periosteal apposition and improved bone structure in the loaded humerus of pre-, compared to peri- or post pubertal girls, aged 8 to 17 years. Similarly, Petit et al.¹⁷ demonstrated that a seven-month jumping program in early-pubertal girls (aged 10.5 years), but not prepubertal (aged 10.1 years), responded more favorably to the intervention, with significantly greater increases in aBMD, CSA, estimated mean cortical thickness and section modulus of the femoral narrow neck than controls. It is unknown if

this relationship between loading and the changes in bone geometric properties can be detected in younger females, as young as four years of age.

We recently demonstrated that young female gymnasts, four to eight years of age, gained significantly more lumbar spine aBMD than controls after two years since initiating training (Laing et al., in preparation). The present study was conducted in a prepubertal sample (i.e., Tanner stage I for breast and pubic hair development throughout two years) from the same study to determine the influences of the initial years of gymnastics training on conformational changes of PF using the hip structural analysis (HSA) program.¹⁹ This study attempted to answer two questions: 1) Will differences be observed in the structural properties of bone within gymnast and control groups over two years? and 2) Will gymnasts who advance to a higher competition level demonstrate the greatest improvements in strength indices of the PF?

METHODS

Design and study participants. A 24-month quasi-experimental, prospective design was used to examine female children aged four to eight years from the Athens, Georgia area. Subjects were participating in a larger ongoing study assessing the initial years of gymnastics training on bone mineral accrual, for which they and their parents gave consent approved by The University of Georgia Institutional Review Board for Human Subjects. Young females were selected based on the criteria that they did not previously participate in organized physical activity (or had limited participation <12 weeks) before beginning their first gymnastics class or non-gymnastic activity. Upon entry into the study, all participants were apparently healthy (reporting no history of disease or conditions known to affect bone metabolism, e.g., rickets, growth hormone

deficiency, use of glucocorticoid medications), and had no evidence of secondary sexual characteristics (i.e., using Tanner staging for breast and pubic hair development) as assessed by a physician.²⁰ At baseline, participants electing to enroll in recreational gymnastics (GYM; n = 31) were compared to a group of control children (CON; n = 31) participating in nongymnastic activities or no activities. Individuals in each group were match-paired based on race, age (± 1 year), height (5 cm), weight (3.5 kg), and sexual maturation (Tanner stage I for both breast and pubic hair through 24-months). The ethnic distribution of this sample was: 73% Caucasian, 20% African-American, 4% Asian and 3% Hispanic. Gymnasts were enrolled in one of three gymnastics programs in the Athens area, and trained an average of one hour per week at baseline. Those gymnasts who advanced to a higher-competition level (HLG; n=9) were compared to low-level gymnasts (LLG; n=9) using the same matching criteria.

Testing procedures were completed at baseline, 12- and 24-months in the Bone and Body Composition Research Laboratory and University Health Services at The University of Georgia. On the day of testing, radiographs, sexual maturation ratings, anthropometric measures, and dual-energy x-ray absorptiometry procedures were performed. In addition, subjects and their parent(s) were instructed on the home completion of 3-day diet records.

Anthropometry. All methods for assessment of anthropometric measures were derived from corresponding techniques listed in the Anthropometric Standardization Reference Manual.²¹ Weight of each subject wearing minimal clothing (i.e., dressed in gym shorts and top, but without shoes), was measured to the nearest 0.25 kg using a calibrated double-beam balance scale (Fairbanks Scales, Kansas City, MO). Stature was

measured without shoes to the nearest 0.10 cm using a wall-mounted stadiometer (Novel Products, Inc., Rockton, IL). Sitting height was measured with each subject seated on a box 50 cm in height using the same stadiometer, and estimations for leg length were calculated using height minus sitting height. In our laboratory, one-way random effects model, single measure ICCs were computed for body weight ($R = 0.99$), standing height ($R = 0.99$), and sitting height ($R = 0.97$) in females aged six to 10 years of age ($n = 10$) who were measured by the same technician twice in a two-week period. The coefficient of variation (CV) for test-retest measures using the same children are 0.4, 1.4, and 0.9% for height, body weight and sitting height, respectively. Stature, body weight and body mass index (BMI; kg/m^2) were plotted for each child's age on growth and BMI charts²² to determine the percentiles associated with each measure and to track changes over time.

Skeletal age assessment. Radiographs of the left hand-wrist were obtained by a qualified radiologist. Skeletal age was assessed using the Fels method.²³ The maturation indicators used for the Fels method have been shown to be both valid and reliable in growing children.^{23, 24} From our overall sample, radiographs from randomly selected participants ($n = 12$) were re-assessed approximately four months after the initial assessments were made. The ICC between assessments was $R = 0.99$, and standard errors of the estimate for skeletal age ranged from 0.26 to 0.29 years in initial and replicate assessments. Furthermore, the mean difference between assessments of skeletal age was -0.01 ± 0.12 year, indicating a high degree of reproducibility.

Sexual maturation rating. Sexual maturation ratings were conducted annually by a physician experienced in performing evaluations using a modified version of Tanner's Stages of Sexual Development rating scale (stages I to V for breast and pubic

hair development).²⁰ In our laboratory, one-way random effects model, single measure ICCs revealed perfect agreement ($R = 1.0$) for both breast and pubic hair assessments in females six to 10 years of age ($n = 10$) from the present study evaluated twice in a two-week period.

Dietary intake measures. Dietary intake was estimated using 3-day diet records distributed to each participant for home completion (two weekdays and one weekend day). Parents and children were instructed by a registered dietitian on the accurate completion of 3-day diet records using a 24-hour recall. After inclusion of dietary calcium intake from supplements, data were analyzed using Food Processor Nutrition and Dietary Analysis System (ESHA Research, Salem, OR). The 3-day diet record has been shown to be a valid instrument for use in children.²⁵ In our laboratory, a one-way random effects model, average measure (i.e., three days) ICCs were calculated for calcium intake ($R = 0.71$) in female children six to 10 years of age ($n = 10$) who completed 3-day diet records twice in a two-week period.

Physical activity assessment. Physical activity was quantified objectively using accelerometry (Model # 7164, Computer Science Applications; CSA/MTI, Fort Walton Beach, FL). Accelerometers were encased in a zippered pouch worn by each subject at the waist (on the midaxillary line) for three days (two week days and one weekend day). Parents were instructed to complete a data-recording sheet indicating the periods of time when the monitor was fastened or removed from the child. Data were recorded by the CSA device during one-minute epochs. Three-day averages for activity counts per minute were generated for each subject. Janz et al.²⁶ and Trost et al.²⁷ reported acceptable reliability data ($r = 0.69$ and 0.70 , respectively) from three to five-day averages in young

children. The CSA monitor has also been shown to be a valid device for the assessment of children's physical activity using energy expenditure measured by calorimetry ($r = 0.87$),²⁸ and heart rate recordings ($r = 0.50$ to 0.74).²⁹ Because the coaches in gymnastics and other organized sports activities discouraged the use of accelerometers during classes for safety reasons, we instructed each child in the gymnast and control groups to remove the monitor during participation in organized activities, so that only leisure time (i.e., not organized) physical activity would be recorded.

Bone densitometry. Non-dominant PF scans were obtained using dual energy x-ray absorptiometry (DXA; QDR-1000W, Hologic Inc., Waltham, MA). Body composition [body fat (g), fat-free soft tissue mass (FFST; g) and percent fat (%FAT)] was estimated from the total body scans, using Hologic Inc. Pediatric Software. Subjects wore light clothing and removed all metal items prior to the scans. Quality assurance for DXA was carried out by daily calibration against the standard phantom provided by the manufacturer. A lumbar spine phantom containing calcium hydroxyapatite and epoxy sections embedded in a lucite cube (Hologic x-caliber anthropometric spine phantom, model DPA/QDR-1) was scanned each morning prior to testing. A CV of 0.27% was observed in our laboratory from 365 scans of the lumbar spine phantom over a five-year period. Quality control for soft tissue measurements was assured by concurrently scanning (with each total body scan) an external three-step soft tissue wedge composed of different thickness levels of aluminum and lucite, calibrated against stearic acid (100% fat) and water (8.6% fat; Hologic, Inc.). Test-retest measurements using DXA in young females five to eight years of age ($n = 10$) scanned twice during a one-week period demonstrated acceptable reliability for the PF (CV = 1.6%; ICC = 0.98).

Hip structural analysis. Structural properties of the PF were assessed using the HSA program.¹⁹ Using this program, three narrow regions within the PF are analyzed corresponding to 5 mm cross-sectional components of bone, and include the narrow neck, intertrochanteric and shaft regions (Fig. 4.1). HSA measures both the aBMD of these regions and their structural geometry. The narrow neck region is placed across the narrowest segment of the femoral neck, the intertrochanteric region along the bisector of the neck-shaft angle and the shaft is placed 2 cm distal to the midpoint of the lesser trochanter. For each region, the distribution of bone mass across the bone is extracted, and structural properties are derived. Key outcome variables for analyses are: cross sectional area, cross sectional moment of inertia, subperiosteal width, section modulus, endosteal diameter, and average cortical thickness. Descriptions of these outcome variables are listed in Table 4.1. Both narrow neck and shaft regions are modeled as circular annuli, while an asymmetric ellipse is assumed for the intertrochanteric region. The intertrochanter model assumes 50/50 proportion of cortical/trabecular bone while the narrow neck region assumes a 60/40 proportion. This method was originally validated in cadaveric bone using varying loads at the PF to determine resistance to bending and breaking strength ($r \geq 0.99$).¹⁹ Precision in longitudinal analyses was determined on 10 scans, three times, with CV ranges of 0.1%-1.2%.

Statistical analyses. Data were analyzed using the Statistical Analysis System (SAS, version 8.2, SAS Institute, Cary, NC). Data were checked for normality and cases were excluded in the instance of outliers (i.e., extreme cases ± 3 SD from the mean). Descriptive statistics (mean \pm SD) were summarized for all variables. Baseline paired t -tests were performed to examine group differences in structural properties. Matched-pair

repeated measures analysis of covariance (RM-ANCOVA) was used for statistical interpretation of the data. The resulting RM-ANCOVA models were used to model the post-baseline responses of the participants, and covariates were used to control for differences among the study groups at baseline. Covariates were selected based on statistical analysis and established relationships between these variables and the growing skeleton, and include body size (baseline weight and height) and composition (the ratio of FFST to body weight), maturity (baseline skeletal age) and dietary intake (baseline calcium intake).³⁰⁻³³ For prospective analytical comparisons, adjusted values based on RM-ANCOVA are used and statistical relationships are reported based on the independence or dependence on group interactions with other covariates. For example, ‘true’ group x time interactions are reported when results are not dependent upon other covariate interactions. Secondly, group x time interactions that depend on various levels of the covariates are reported.

In order to determine if high-level gymnasts demonstrate greater changes in structural properties compared to low-level gymnasts, our sample of gymnasts was divided into high- vs. low-level based on total hours of gymnastics participation. A histogram of raw hours of gymnastics classes (over the 24-month testing period) was completed and revealed a bimodal frequency distribution, where two distinct groups emerged: one lower (<100 hours; mean=63 hours over two years; average of one hour per week) and one higher activity (>100 hours; mean=259 hours over two years; average of eight hours per week). An alpha-level of 0.05 was chosen to identify statistical significance.

RESULTS

Select participant characteristics at baseline and two years are displayed in Table 4.2. There were no differences in chronological or skeletal age, anthropometric measures, leisure time physical activity, calcium intake or body composition between groups at baseline and over two years. No statistically significant group x time interactions existed for these variables, however, all participants had similar increases in sitting height, weight, BMI and fat mass (time effect; $p < 0.01$), when holding initial values as covariates. All participants were classified as Tanner stage I for both breast and pubic hair development at baseline and throughout the two-year study.

Baseline and two-year unadjusted values for geometric structural properties of the PF are illustrated in Table 4.3. No differences were observed between GYM and CON for any of the structural variables at baseline. At two years, significant ($p = 0.05$ for endosteal diameter) and moderate ($p = 0.06$ for subperiosteal width) group effects were observed at the narrow neck, where $CON > GYM$. In addition, GYM had a significantly greater value for centroid position compared to CON ($p < 0.05$) at two years.

After adjusting for baseline calcium intake, height, weight, skeletal age, and FFST:weight, group x time interactions existed at the narrow neck where $GYM > CON$ for CSA ($p = 0.06$), CSMI ($p = 0.02$) and section modulus ($p = 0.04$). Conversely, subperiosteal width ($p = 0.08$) and endosteal diameter ($p < 0.01$) increased more in CON vs. GYM. The interactions observed for changes in CSA, CSMI, section modulus and subperiosteal width all depended on initial weight. At the intertrochanteric region, group x time interactions existed where $GYM > CON$ for CSMI ($p = 0.05$) and section modulus ($p = 0.04$), whereas subperiosteal width increased more in CON vs. GYM ($p = 0.03$). The

interactions observed for changes in CSMI and section modulus both depended on initial weight, whereas the changes in subperiosteal width depended on initial height. No separate group or time effects were present for the shaft. No PF geometric differences were observed at the narrow neck, intertrochanteric or shaft regions for HLG vs. LLG.

DISCUSSION

The structural properties of bone were examined following two years of gymnastics training in prepubertal females. Our primary finding was that recreation-level artistic gymnastics initiated at a young age in novice females did not promote significant changes in structural properties in the narrow neck or intertrochanteric regions of the PF independent of initial height and weight. However, gymnasts had greater increases in CSA, CSMI and section modulus at the narrow neck compared to controls, depending on initial weight, where those gymnasts who were heavier, had the greatest PF strength advantage over controls. Similarly, at the intertrochanteric region, gymnasts had greater increases in CSMI and section modulus, also depending on initial weight. Controls had greater increases in subperiosteal width depending on initial weight (at the narrow neck) and height (at the intertrochanter). Endosteal diameter, however, increased to a significantly greater extent in the controls compared to the gymnasts, independent of initial growth variables.

The sport of gymnastics produces bending and torsional strains on the skeleton that lead to the high aBMD values commonly seen in gymnasts vs. nongymnasts.^{3, 4, 10, 34} In our ongoing project evaluating the effects of gymnastics training on bone mineral accrual in young females, we demonstrated a significant gain in total PF aBMD in the gymnasts, 1.5% beyond values achieved by controls ($p < 0.05$) (Laing et al., in review).

Petit and coworkers,¹⁷ who also discovered an increase in aBMD at the PF in jumpers vs. controls, indicated that the observed increase in aBMD could be the result of one or several processes taking place in the PF: *greater* subperiosteal bone formation or *less* medullary canal (i.e., endocortical) expansion in the gymnasts vs. controls, or *greater* medullary canal contraction in controls vs. gymnasts. Upon examining these structural components in our same female gymnasts and controls, it was hypothesized that we would observe greater aBMD and CSA values in the narrow neck, intertrochanter and shaft regions of the PF in gymnasts over time, comparable to the aBMD differences observed at the total PF.

Structural differences at the shaft region of the PF were not observed between gymnasts and controls. These findings were similar to those presented by Petit et al.,¹⁷ and in contrast to observations by Bass et al.¹⁰ and Faulkner et al.¹⁶ (Table 4.4), who found that elite gymnasts had higher values for aBMD, average cortical thickness, section modulus, CSMI, subperiosteal width and CSA, and lower values for endosteal diameter compared to controls. One possible reason for lack of differences at the shaft from our study and those by Petit et al.¹⁷ is that the exercise protocols were not as strenuous as the training schedule of the elite gymnasts. The gymnasts in our study were enrolled in recreation-level classes for an average of one hour per week, performing a combination of jumping and tumbling activities. The jumping protocol followed in the Petit et al.¹⁷ study consisted of 12 minutes of jumping three times per week (for a total of 36 minutes per week). In contrast, elite-level gymnasts studied in the cross-sectional observations by Faulkner et al.¹⁶ and Bass et al.¹⁰ trained competitively at the national level, up to 36 hours per week, and had been competitive in the sport for up to five years. This may

explain why detectable geometric structural differences were observed at the shaft region between gymnasts and controls in these studies. It is therefore likely that a greater strain magnitude, rate or duration of activity in the elite gymnasts may have been necessary to achieve a significant osteogenic response at the primarily cortical shaft of the femur, beyond what was achieved by recreational gymnasts in our study.

Part of the explanation for the bone response in the observations by Bass et al.¹⁰ and Faulkner et al.¹⁶ may be related to a higher proportion of fat-free mass. Fat-free soft tissue mass is one of the most powerful determinants of aBMD acquisition (as much as 60% of the variance in adolescent girls).³⁵ Skeletal muscle, the primary component of FFST, exerts a force on bone during muscle contraction.³⁶ In a recent study of adolescent gymnasts, it was suggested that the increases in bone mineral measures were likely due to the amount of lean mass present.⁴ Compared to age-matched controls, prepubertal elite gymnasts, training a minimum of 15 hours per week, had significantly greater size-adjusted strength indices (CSMI, and section modulus) at the narrow neck and shaft regions, using the hip structural analysis program.¹⁶ Yet, when adjusted for lean body mass, these differences no longer existed. Over time, gymnasts in the present study did not demonstrate significant changes in lean body mass, fat mass or percent fat. In our sample, the average relative fat-free mass (i.e., the percent of fat-free soft tissue mass to weight) was approximately 69% in both gymnast and control groups. Faulkner et al.¹⁶ demonstrated that gymnasts had 78% relative fat-free mass vs. 68% in controls. Although Bass et al.¹⁰ did not provide absolute values for body weight, they did report that gymnasts had 10% greater lean mass than the controls. We speculate that due to the young age of the children or perhaps the maneuvers performed in this community-based

intervention, the children were not able to gain a sufficient amount of lean tissue or the forces necessary to elicit structural changes in the PF, independent of initial height and weight measures. When we observed gymnasts who were competing at a high-level (albeit not elite), compared to low-level gymnasts, no significant differences were observed at any region measured.

The children examined in the studies used for comparison (Table 4.4), although classified as prepubertal, were between the ages of 10 to 12 years. It is therefore possible that the young age of our participants (mean age 5.8 years) may be limiting their ability to achieve structural changes with exercise at the narrow neck and intertrochanteric regions without dependency on height or weight. A recent study by Specker et al.¹⁸ demonstrated that conformational changes could be induced by exercise and calcium intervention in three to five year old children. However, this study investigated changes at the peripheral tibia and not the PF. Perhaps with the examination of bone sites apart from the PF, the benefits of gymnastics training (such as that observed at the lumbar spine in our larger study) would be detected.

Our result that gymnasts had increased CSA, CSMI and section modulus at the narrow neck over time compared to controls is consistent with results from studies presented in Table 4.4. Based on the results presented by Petit et al.,¹⁷ the majority of structural changes occurred in the narrow neck, where the jumping protocol promoted higher rates of aBMD, CSA, section modulus and estimated mean cortical thickness in early pubertal girls (CSMI was not reported). No differences were observed between groups in their prepubertal sample. Our results indicate that estimated mean cortical thickness or aBMD of the narrow neck did not change following the initial two years of

gymnastics activity. Control participants had greater increases in subperiosteal width, indicating more bone formation, but this group also demonstrated significantly greater endosteal diameter, indicating bone resorption on that surface. These two processes likely resulted in the slightly higher CSA values observed over time in the gymnasts. Since CSA changed slightly over time at the narrow neck (depending on weight), this indicates that periosteal bone formation may have been occurring at a greater rate beyond that experienced by the controls. Faulkner et al.¹⁶ demonstrated that CSA and section modulus were significantly greater for gymnasts at the narrow neck region. These results suggest that the PF in gymnasts is adapted for the loading conditions imposed by this activity. Consistent with these results, the endocortical diameter and subperiosteal width at the narrow neck were smaller in our sample of gymnasts vs. controls.

There are limitations to attempting to assess three-dimensional structure using two-dimensional imaging techniques. Densitometry-derived measures of BMC and aBMD are good predictors of future osteoporotic fractures,³⁹ however the inability of these measurements to distinguish between the contribution of bone size and bone mass makes predictions of strength difficult in growing children. For example, HSA precision can be greatly influenced by subject positioning on the DXA.⁴⁰ The assumption of the relative distribution of trabecular and cortical bone in children has not been validated. However, a key strength to this study was our prospective design, where assessment of change in structural properties could be observed over two years in a carefully matched group of prepubertal children.

CONCLUSION

While we observed that gymnasts had moderately higher CSA, CSMI and section modulus values at the narrow neck compared to controls, these interactions were dependent upon initial weight, where those gymnasts who were heavier, had the greatest strength advantage at the PF over controls. At the narrow neck, controls had greater increases in subperiosteal width (dependent on weight) compared to gymnasts. We detected similar differences at the intertrochanter, where gymnasts had greater increases in CSMI and section modulus (depending on weight) and controls had greater increases in subperiosteal width (depending on height). We did not detect differences over time in aBMD, or average cortical thickness within the gymnast or control groups for the three measured regions. Interestingly, controls demonstrated greater increases in endosteal diameter at the narrow neck, indicating increased resorption on the endosteal surface.

Based on these results, increases in aBMD at the total proximal femur (observed in our ongoing study) did not necessarily translate into improvements in structural or strength properties using HSA. Since we did not observe changes in PF structural properties in the high-level gymnasts vs. the low-level gymnasts, it is likely that even the high-level maneuvers performed by the young gymnasts in this study were not great enough to elicit a geometric conformation of bone at the proximal femur. It is possible that the positive effect of gymnastics participation on estimated bone strength in the femur will emerge as the girls become more developmentally mature. It may also be possible to observe positive effects from gymnastics training at other skeletal sites, such as the proximal tibia.

ACKNOWLEDGMENTS

This research was supported by National Institute on Child Health and Human Development grant 1 RO1 HD 35592-01A1. Additional financial support was provided to Emma M. Laing through the American Dietetic Association and The University of Georgia College of Family and Consumer Sciences.

TABLES AND FIGURES

TABLE 4.1. Description of outcome variables produced using hip structural analysis.¹⁹

Neck length (cm)	Distance from the center of femoral head to intersection of neck and shaft axes
aBMD (g/cm ²)	Areal Bone mineral density
CSA (cm ²)	Cross sectional area: index of axial strength; equivalent to the amount of cortical bone in the cross-section, not including the trabecular and soft tissue spaces
CSMI (cm ⁴)	Cross-sectional moment of inertia; a measure of the cross-sectional shape of the bone around the centroid used to determine the bending and torsional characteristics of bone; value is calculated as the 4 th power of the radius
Subperiosteal width (cm)	Diameter of the bone width computed as the blur-corrected width of the mass profile
Section modulus (cm ³)	Index of bending strength; for the narrow neck and shaft regions is taken as the [CSMI/ 1/2 subperiosteal width]; in the intertrochanteric region [CSMI/ distance from the lateral margin to the region centroid]
Endosteal diameter (cm)	Estimate of inside diameter of cortex
Average cortical thickness (cm)	The subperiosteal width minus [endocortical diameter / 2]
Centroid position (cm)	Distance from centroid to medial margin / bone subperiosteal width

TABLE 4.2. Participant characteristics at baseline and two years.

	Baseline						Two Years					
	Gymnasts (n=31)			Controls (n=31)			Gymnasts (n=31)			Controls (n=31)		
<i>Age and Anthropometrics</i>												
Age (yr)	5.8	±	1.26	5.7	±	1.33	7.9	±	1.26	7.8	±	1.32
Height (cm)	114	±	10.3	113	±	10.8	127	±	10.0	127	±	10.6
Weight (kg)	20.7	±	3.60	20.6	±	3.79	27.2	±	5.57	27.5	±	6.91
BMI (kg/m ²)	16.0	±	1.58	15.9	±	1.83	16.8	±	2.38	16.9	±	3.27
Sitting height (cm)	62.1	±	4.03	61.9	±	3.58	68.8	±	3.62	69.0	±	3.09
Leg length (cm)	51.5	±	4.36	51.5	±	4.19	58.2	±	6.24	58.1	±	6.18
Skeletal age (yr)	6.0	±	1.21	5.9	±	1.29	8.2	±	1.30	8.0	±	1.50
<i>Physical Activity</i>												
Accelerometer cts/min	753	±	129	699	±	205	781	±	386	828	±	344
<i>Dietary Intake</i>												
Calcium (mg)	849	±	332	889	±	392	954	±	458	890	±	333
<i>Body Composition</i>												
Fat mass (g)	4802	±	2037	4850	±	2243	6840	±	3822	7120	±	4724
Lean mass (g)	14431	±	2122	14241	±	2223	18504	±	2510	18329	±	2774
Fat (%)	23.5	±	6.66	23.8	±	7.20	24.6	±	8.70	25.1	±	9.18
Lean mass: weight (%)	70.0	±	0.06	0.69	±	0.06	0.69	±	0.08	0.68	±	0.09
<i>Tanner Stage</i>												
Breast	1.00	±	0.00	1.00	±	0.00	1.00	±	0.00	1.00	±	0.00
Pubic hair	1.00	±	0.00	1.00	±	0.00	1.00	±	0.00	1.00	±	0.00

Values are unadjusted means ± SD

TABLE 4.3. Proximal femur structural properties at baseline and two years.

	Baseline				Two Years			
	Gymnasts (n=31)		Controls (n=31)		Gymnasts (n=31)		Controls (n=31)	
<i>Narrow Neck Region</i>								
Neck length (cm)	3.87	± 0.69	3.66	± 0.45	3.91	± 0.52	3.91	± 0.75
aBMD (g/cm ²)	0.561	± 0.07	0.547	± 0.07	0.555	± 0.06	0.538	± 0.05
Cross sectional area (cm ²)	1.17	± 0.18	1.11	± 0.21	1.11	± 0.17	1.15	± 0.19
CSMI (cm ⁴)	0.460	± 0.14	0.410	± 0.14	0.408	± 0.13	0.468	± 0.17
Subperiosteal width (cm)	2.19	± 0.23	2.13	± 0.20	2.11	± 0.22 [†]	2.23	± 0.26
Section modulus (cm ⁴)	0.402	± 0.09	0.373	± 0.10	0.371	± 0.09	0.401	± 0.11
Endosteal diameter (cm)	1.98	± 0.23	1.92	± 0.19	1.90	± 0.22*	2.02	± 0.26
ACT (cm)	0.11	± 0.01	0.10	± 0.01	0.11	± 0.01	0.10	± 0.01
Centroid position (cm)	0.51	± 0.01	0.50	± 0.01	0.51	± 0.01*	0.50	± 0.01
<i>Intertrochanteric Region</i>								
aBMD (g/cm ²)	0.573	± 0.08	0.558	± 0.08	0.561	± 0.06	0.555	± 0.07
Cross sectional area (cm ²)	1.74	± 0.41	1.67	± 0.34	1.74	± 0.37	1.74	± 0.38
CSMI (cm ⁴)	1.451	± 0.62	1.402	± 0.49	1.633	± 0.81	1.667	± 0.81
Subperiosteal width (cm)	3.19	± 0.46	3.13	± 0.32	3.24	± 0.50	3.28	± 0.52
Section modulus (cm ⁴)	0.913	± 0.33	0.852	± 0.23	0.937	± 0.32	0.941	± 0.32
Endosteal diameter (cm)	2.78	± 0.42	2.75	± 1.29	2.85	± 0.46	2.68	± 0.85
ACT (cm)	0.20	± 0.03	0.20	± 0.03	0.19	± 0.02	0.19	± 0.03
Centroid position (cm)	0.49	± 0.01	0.49	± 0.02	0.49	± 0.01	0.49	± 0.01
<i>Shaft Region</i>								
aBMD (g/cm ²)	0.689	± 0.10	0.661	± 0.10	0.662	± 0.07	0.663	± 0.09
Cross sectional area (cm ²)	1.35	± 0.26	1.32	± 0.26	1.34	± 0.25	1.34	± 0.25
CSMI (cm ⁴)	0.538	± 0.21	0.537	± 0.19	0.565	± 0.22	0.570	± 0.22
Subperiosteal width (cm)	2.06	± 0.22	2.09	± 0.21	2.12	± 0.26	2.12	± 0.23
Section modulus (cm ⁴)	0.496	± 0.15	0.492	± 0.14	0.505	± 0.14	0.513	± 0.15
Endosteal diameter (cm)	1.58	± 0.22	1.64	± 0.21	1.67	± 0.24	1.67	± 0.22
ACT (cm)	0.24	± 0.04	0.23	± 0.04	0.25	± 0.02	0.23	± 0.03
Centroid position (cm)	0.51	± 0.01	0.51	± 0.01	0.51	± 0.01	0.51	± 0.01

Values are unadjusted means ± SD

*Significant difference (p < 0.05) between Gymnasts and Controls

[†]Non-significant difference (p = 0.06) between Gymnasts and Controls

aBMD; Areal bone mineral density

CSMI; Cross sectional moment of inertia

ACT; Average cortical thickness

TABLE 4.4. Description of results from geometric assessments in children where high-load bearing activities were performed.

<i>Narrow Neck</i>	<i>aBMD</i>	<i>ACT</i>	<i>Endosteal Diameter</i>	<i>Section Modulus</i>	<i>Subperiosteal Width</i>	<i>CSMI</i>	<i>CSA</i>
Current Study	No change	No change	C > G	G > C ^w	C > G ^w	G > C ^w	G > C ^w
Faulkner et al. ¹⁶	G > C	N/A	C > G	G > C	C > G	No difference	G > C
Petit et al. ¹⁷	I > C	I > C	C > I*	I > C	C > I*	N/A	I > C
<i>Inter-Trochanter</i>							
Current Study	No change	No change	No change	G > C ^w	C > G ^h	G > C ^w	No change
Petit et al. ¹⁷	I > C	N/A	C > I	I > C*	C > I*	N/A	I > C*
<i>Shaft</i>							
Faulkner et al. ¹⁶	G > C	N/A	No difference	G > C	G > C	G > C	G > C
Bass et al. ¹⁰	G > C	G > C	C > G	N/A	No Difference	N/A	N/A

Values are statistically significant relationships

*Non-significant interaction

^wInteraction depends on weight; G > C for heaviest girls

^hInteraction depends on height; C > G for tallest girls

G= Gymnasts; C = Controls; I = Intervention group

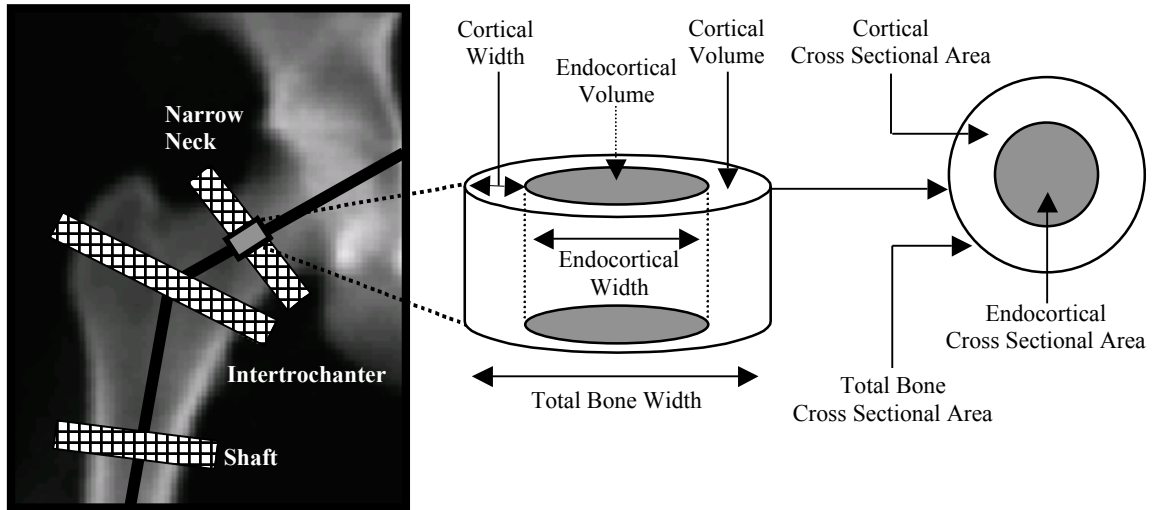
aBMD; areal bone mineral density

ACT; average cortical thickness

CSMI; cross section moment of inertia

CSA; cross sectional area

FIGURE 4.1--Hip structural analysis program regions of the proximal femur obtained using densitometry scan.



Increases in aBMD can be due to increasing subperiosteal bone formation, less medullary/endocortical expansion or greater medullary canal contraction. Adapted from Modlesky and Lewis¹²

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CHAPTER 5

SUMMARY AND FUTURE DIRECTIONS

The present study was conducted to determine the influences of the initial years of artistic gymnastics training on bone in prepubertal children with essentially no organized physical activity experience prior to the onset of training. Results from the study presented in Chapter 3 demonstrated that children electing to enroll in gymnastics activity were significantly shorter, lighter and leaner, and had lower bone mineral values compared to controls. However, over two years, gymnasts had significantly greater gains in lumbar spine aBMD (3.5%) and radius bone area (3.6%) compared to controls. Similarly, it was demonstrated in an individually race-, age-, height- and weight-matched prepubertal sample of gymnasts and controls (n=31 per group), that gymnasts had significantly higher rates of lumbar spine and total proximal femur aBMD accrual, up to 2.7 and 1.5%, respectively, beyond the observed changes in the controls. Additionally, those gymnasts who advanced to a higher competition level had greater aBMD gains at the lumbar spine (3.9%) and radius (3.0%) compared to lower level competitors. Leisure time physical activity and dietary intakes of calcium and vitamin D were not different between the groups or over time.

The study presented in Chapter 4 was conducted in a matched sample of prepubertal gymnasts vs. controls, individually paired for age, height, weight and sexual maturation (i.e., Tanner stage I for breast and pubic hair development throughout two years) to determine the influences of the initial years of gymnastics training on

conformational changes of the proximal femur, using the hip structural analysis (HSA) program.¹ Over two years, gymnasts did not differ from controls in strength variables at the shaft region of the proximal femur. At the narrow neck and intertrochanteric regions, however, gymnasts demonstrated greater increases in cross-sectional moment of inertia and section modulus compared to controls. Furthermore, gymnasts had greater increases in CSA compared to controls at the narrow neck. However, the improvements that were observed depended on initial height and weight, where those gymnasts who were taller, heavier or more developmentally mature, had the greatest advantage over controls in improving the structural quality of these regions. Controls had greater increases in endosteal diameter and subperiosteal width than gymnasts at the narrow neck, and greater increases in subperiosteal width at the intertrochanteric region. The overall findings from these studies suggest that the initial two years of recreational artistic gymnastics training in prepubertal children increases aBMD at the lumbar spine and bone area of the radius beyond those observed in controls, however only modest differences are observed in the structural properties of the narrow neck and intertrochanteric regions within the proximal femur, as assessed by HSA. It may be possible that the positive effects of gymnastics participation on estimated bone strength in the proximal femur will emerge as these young females advance in maturity.

The results presented are important with respect to recreational-level gymnastics training and pediatric bone. However, they lead to more questions regarding skeletal responses to exercise in the context of maturational differences. For instance, not all young gymnasts persist in the sport. How do the size and maturity of those who drop out compare to those who persist in the sport? Will the differences in lumbar spine aBMD be

maintained in these children if they withdraw from gymnastics training? If so, is there a minimum level of stimulus required to maintain the gains? Will those who remain in the sport continue to gain bone mineral at a greater rate than nongymnast controls? If so, is there a level of training that is most advantageous? Few data exist with respect to this issue. Observations in adult, retired athletes²⁻⁴ provide most of the evidence that benefits of athletic participation during youth leads to higher levels of aBMD, years after the activity was ceased. Fuchs et al.⁵ demonstrate that the skeletal benefits of jumping exercise in children can be maintained nine to 12 months after withdrawal from the intervention, however, these observations are made after a relatively short duration from when the activity was ceased, and do not examine skeletal changes within the various stages of maturation with growth in the same individuals. Longer-term prospective investigations are needed to address the issue of maintenance of bone mass from the prepubertal years into young adulthood.

In conclusion, it may be possible that dietary and activity habits shown to benefit the skeleton in childhood track into adult years.⁶ According to Hui et al.,⁷ a change of one standard deviation in bone mass may alter the risk of fracture by as much as 120%. Previous studies have determined that persons who consume greater quantities of calcium⁸ or engage in weight-bearing physical activities²⁻⁴ early in life, have greater bone mineral in adulthood compared to inactive individuals. In the study by Kirchner et al.,² the higher bone mass in retired gymnasts, 9 to 22% beyond the observed values in controls, gives further evidence to the idea that there is a residual effect on the maintenance of bone mass into adulthood. Based on the results of studies presented here, a 3 to 4% increase in lumbar spine aBMD over two years was observed in prepubertal

females who initiated the sport of gymnastics. While slight differences in bone geometric properties were observed between gymnasts and controls, there is the potential for more profound differences to emerge during early puberty. If such geometric and mineral properties of bone are able to permanently alter the skeleton through gymnastics training, these children have the potential for a decreased risk of fracture and a benefit to long-term bone health.

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APPENDICES

APPENDIX A
Recruitment Flyer

GET PAID TO PLAY...

ENROLL YOUR DAUGHTER TODAY !!!

~THE UGA BONE STUDY~



Benefits:

☺ **\$520** or

☺ **\$20** for each referral who enrolls in the study (not in your immediate family)

☺ **FREE** dietary and physical activity analyses

☺ **FREE** bone and body composition analyses

☺ Girls between the ages of 4 and 8 are invited to participate in the study.

☺ If your daughter has never participated in an organized sport, or has participated for one quarter or less, she may be eligible.

For More Information and eligibility guidelines please call Emma at: (706) 542-4918 or email at bones@uga.edu

APPENDIX B

Assent and Consent Forms

Assent Form (for children)

I _____ agree to take part in a study about bone health and growth.

I do not have to be in this study if I do not want to be. I have the right to leave the study at any time without giving any reason, and without penalty.

I will have pictures taken of my bones. During one set of pictures I will lie on a table for approximately one hour. I will take short breaks between the different pictures that are taken. During another set of pictures I will place my arm on a box for about 5 minutes.

I will have my height measured against a wall and my weight measured on a scale.

I will answer questions about the activities that I participate in, the foods that I eat and how I perceive the shape of my body.

I will wear a little pouch during two weekdays and one weekend day. The pouch will measure how much I move around.

My parent and I will write down what I eat during two weekdays and one weekend day.

Some of the questions may cause me to be uncomfortable. I may skip any question that I do not wish to answer.

My answers and any information about me will be kept confidential. This means that the researchers will not use my name. It also means that my responses to questions and any information about me will not be shared with anyone else.

If I have any questions about the research I can call the researchers and they will answer all questions I have. The researchers are Dr. Lewis, Mr. Modlesky, Ms. Laing, Dr. O'Connor and Dr. Baile. Their phone number is 706-542-4918.

I will sign both copies of this form. I will keep one for myself and I will return the other to the researchers.

Signature of Child Participant Date

Signature of Researcher Date

For questions or problems about your rights please call or write: Chris A. Joseph, Ph.D., Human Subjects Office, University of Georgia, 606A Boyd Graduate Studies Research Center, Athens, Georgia 30602-7411; Telephone (706) 542-6514; E-Mail Address: IRB@uga.edu.

CONSENT FORM (PARENT/CHILD)

I _____ agree to participate and give consent for my child, _____, to participate in the research titled "Determinants of bone health in young female gymnasts," which is being conducted by Richard D. Lewis, Christopher M. Modlesky, Emma Laing and Dr. Clifton A. Baile of the Department of Foods and Nutrition and Dr. Patrick J. O'Connor of the Department of Exercise Science of the University of Georgia. Dr. Lewis and Mr. Modlesky may be reached in room 279 Dawson Hall at 542-4901 or 542-4918. Dr. O'Connor may be reached in room 115L of the Ramsey Center or at 542-4382. I understand that the participation of my daughter is completely voluntary. I can withdraw consent at any time without penalty and have the results of the participation, to the extent that which it can be identified as my child's, returned to me, removed from the research records, or destroyed.

The following points have been explained to me and my daughter:

1) The reason for the research is to study the impact of gymnastics activity initiated at an early age on bone and growth in children. The benefits that my daughter and I can expect from participation are the assessment of bone health (bone mineral density), body composition (percentage of body fat and lean mass), dietary intake and growth. In addition, my daughter's gymnastics tuition (up to \$65/quarter) will be paid for the duration of her participation in the study. Tuition will be paid only if all testing sessions are completed for a given time point. All measurements are being used for research purposes only, not medical purposes. However, if abnormalities are found in any measure, I and/or my daughter will be notified and referred to an appropriate health care professional.

2) The procedures are as follows:

a) Testing will be conducted at five different time points: months 0, 6, 12, 18 and 24. At 0, 12 and 24 months three different testing sessions (Session 1, Session 2 and Session 3) will be required, whereas, only Session 3 will be required at 6 and 18 months.

b) The day before testing for Session 1, my daughter will have fasted overnight to obtain accurate urine and blood measures of bone health. On the day of testing for Session 1, my daughter and I will arrive in the Sports Nutrition Lab in Dawson Hall at the scheduled time (early in the morning). Prior to any testing or participation, a consent form will be read to my daughter and I, after which I, my daughter and the researcher will sign the consent form. During the reading of the consent form, my daughter and I will be briefed and familiarized with the testing procedures that will be used during the study (15 minutes). My daughter and I will be given the opportunity to reread the consent form and ask any questions that we may have about the study. Each phase of the study will be explained to my daughter and I throughout testing and we can withdraw from the study at any time. A copy of the consent form will be provided for my spouse to read. Prior to any testing, my daughter and I will be walked through all procedures and reminded that we are free to withdraw at any time. My daughter and I will then be walked to a private room where a Gynecologist/Obstetrician will assess my daughter's pubic hair and breast development, in my presence and in the presence of a female chaperone, to determine level of sexual maturation.

My daughter will be provided with a sterile urine specimen container, walked to the female restroom by a female researcher, instructed on urine collection, allowed to collect her urine in private, and walked back to the Sports Nutrition Laboratory. A trained phlebotomist will then draw approximately 20 mL of blood from my daughter, after which she will be given a snack (15-20 minutes). If a blood sample cannot be obtained after two attempts, no further attempts will be made.

A Research Assistant will then familiarize my daughter and I with the use of an accelerometer (instrument used to assess physical activity) and completion of physical activity diaries. My daughter will wear the accelerometer and keep a physical activity diary for four days (2 weekdays and 2 weekend days) at each time point.

Session 1 will require approximately 90 minutes. Upon completion of Session 1, subjects will be scheduled for Session 2 and Session 3.

c) On the day of testing for Session 2, my daughter and I will arrive at Gilbert Health Center at a predetermined time. To assess bone age a radiograph of the hand/wrist will be conducted by a trained radiologist (30 minutes including waiting time).

d) Upon arrival at the Sports Nutrition Lab for Session 3, my daughter will be asked to complete questionnaires dealing with her body shape perception (approximately 30 minutes).

e) After completion of the questionnaires, my daughter's height, sitting height, leg length and weight, and my height will be measured. My daughter's body composition and bone mineral density will be measured using a QDR 1000/W dual energy X-ray absorptiometer (DXA; Hologic, Inc.). These measurements will require approximately 60 minutes, which includes a small break in between each scan (four scans total). I understand that all DXA measurements will be conducted by a trained laboratory technician or graduate assistant under the supervision of Dr. Richard D. Lewis.

f) After completion of the DXA scans, dietary intake, physical activity and demographic questionnaires will be administered to my daughter and I by a researcher (60 minutes).

g) I understand that when my daughter begins gymnastics training, all gymnastics practices will be documented by videotape. These videotapes will be used to rate the overall gymnastics activity of my daughter.

3) The discomforts or stresses that may be faced during this research are minor discomfort from blood draws, urine collection and sexual maturation ratings. If undue discomfort or stress occurs, my daughter has the right to discontinue the testing at any time.

4) I understand that the only foreseen risk to my daughter is exposure to a small amount of radiation when assessing body composition and bone mineral density with DXA and bone age with radiographs. The scans will give a total maximum radiation dose of 7.5 mR. This dose is very small, as radiation doses from a dental bite-wing film are 334 mR, environmental background is 3.5 mR/week, and chest x-ray films are about 25-40 mR for 2 standard films. Thus the exposure per session is 19-30% of standard chest x-rays. In the event that information from any scan is lost or unusable, no additional scans will be performed.

5) The results of my participation and that of my daughter will be confidential and will not be released in any identifiable form without mine and my daughter's prior consent unless required by law. My signature and that of my daughter's on this form authorizes the use of mine and my daughter's data in group analyses which may be prepared for public dissemination, without breaching my own or my daughter's confidentiality. To accomplish this, my daughter and I will be assigned a four digit subject participation code which will be used on all data collected during mine and my daughter's participation in this research. A master list with mine and my daughter's name and corresponding code number will be kept separate from testing data and locked at all times.

6) The investigator will answer any further questions that I or my daughter may have about this research, either now or during the course of the project.

Signature of Investigator

Date

Signature of Participant

Date

Signature of Parent or Guardian

Date

PLEASE SIGN BOTH COPIES. KEEP ONE AND RETURN THE OTHER TO THE INVESTIGATOR.

Research at the University of Georgia which involves human subjects is carried out under the oversight of the Institutional Review Board. Questions or problems regarding your child's rights as a participant should be addressed to: Chris A. Joseph, Ph.D., Human Subjects Office, University of Georgia, 606A Boyd Graduate Studies Research Center, Athens, Georgia 30602-7411; Telephone (706) 542-6514; E-Mail Address: IRB@uga.edu.

APPENDIX C
Testing Checklist

BONE, GROWTH, AND DIETARY INTAKES IN 4-8 YEAR OLD GIRLS

CHECKLIST - FOLLOW-UP, SPRING 2003

ID Number: _____

<i>initials</i>	<i>date completed</i>	
_____	_____	calendar sign up for DXA's and Questionnaires
_____	_____	sign consent forms
_____	_____	blood draw
_____	_____	urine collection
_____	_____	give food and drink
_____	_____	sexual maturation rating
_____	_____	demographic data questionnaires
_____	_____	anthropometric data sheet
_____	_____	DXA scans
_____	_____	whole body
_____	_____	femur
_____	_____	lumbar spine
_____	_____	radius
_____	_____	physical activity questionnaires
_____	_____	24-hour recall
_____	_____	3-day diet record instructions and sheets
_____	_____	food frequency questionnaire
_____	_____	body image questionnaires
_____	_____	accelerometer
_____	_____	radiograph
_____	_____	final review (Thank You sent)

APPENDIX D

24-Hour Recall

24 HOUR RECALL

Date of Record _____ Subject Code No. _____

DAY OF WEEK TAKE: M T W TH F S SUN (CIRCLE)

Food and Beverage Consumed						
CODE NO.		WHAT DID YOU EAT?	AMOUNT	COOKING METHOD	TIME OF DAY	ACTIVITY WHILE EATING
	Example	EGGS	2 med.	fried	7:30 a.m.	talking with family
	BREAKFAST	Oil	1 tbs.			
	SNACK					
	LUNCH					
	SNACK					
	DINNER					
	SNACK					
	ANY OTHER TIME					

APPENDIX E

Three-Day Diet Record

DIRECTIONS FOR KEEPING A 3-DAY DIET DIARY

Please write down everything you eat (meals, snacks, beverages) for three days on these forms. Please select **TWO WEEKDAYS AND ONE WEEKEND DAY**. Use as much space as you need.

- 1. Write down the date and day at the top of the form.**
- 2. Write down the first foods you ate for that day. Write down:**

- a. The time of day you ate the food(s).
- b. Each food that you ate.
- c. How the food was prepared (baked, boiled, fried, microwaved).
- d. How much you ate (cup, 1/2 cup, pieces, tablespoons, teaspoons).

- 3. It is important to describe each food you eat in detail.**

For example:

Write down brand names for each food you ate if you know them.

Write down the type of milk (whole, 2%, or skim) and bread (white, wheat, etc).

Write down if the food was fresh, frozen, or canned.

If you ate a casserole or a salad, write down the foods/ingredients there were in it and the amounts.

If you add things like butter, jelly, sugar, honey, or cream to foods or beverages, please write them down with the amounts used.

- 4. Do you drink whole _____, 2% _____, 1% _____, or skim _____ milk?**
- 5. Do you use white _____ or whole-wheat _____ bread?**
- 6. What is the complete name and brand of bread that you eat most often?**

- 7. About how many glasses of water do you drink each day? _____**

DAY 1 OF THE DIET DIARY

ID: _____ CHECKED BY: _____

DATE: _____ DAY OF THE WEEK: _____

Did you drink a calcium-fortified beverage today (e.g. Calcium-fortified orange juice) or eat a calcium-fortified food (e.g. Total breakfast cereal)? Yes No

If yes, list all the calcium-fortified beverages/foods, with the BRAND name, and how much you consumed:

Write down everything you eat, beginning with the first thing you have for breakfast. Be sure to include very detailed information such as how the food was prepared, how much you ate, and the brand names.

Time Eaten	Foods Eaten	Preparation Methods	Amount (cup, 1/2 cup, piece, etc)

Please continue on the back of page if necessary.

DAY 2 OF THE DIET DIARY

ID: _____ CHECKED BY: _____

DATE: _____ DAY OF THE WEEK: _____

Did you drink a calcium-fortified beverage today (e.g. Calcium-fortified orange juice) or eat a calcium-fortified food (e.g. Total breakfast cereal)? Yes No

If yes, list all the calcium-fortified beverages/foods, with the BRAND name, and how much you consumed:

Write down everything you eat, beginning with the first thing you have for breakfast. Be sure to include very detailed information such as how the food was prepared, how much you ate, and the brand names.

Time Eaten	Foods Eaten	Preparation Methods	Amount (cup, 1/2 cup, piece, etc)

Please continue on the back of page if necessary.

DAY 3 OF THE DIET DIARY

ID: _____ CHECKED BY: _____

DATE: _____ DAY OF THE WEEK: _____

Did you drink a calcium-fortified beverage today (e.g. Calcium-fortified orange juice) or eat a calcium-fortified food (e.g. Total breakfast cereal)? Yes No

If yes, list all the calcium-fortified beverages/foods, with the BRAND name, and how much you consumed:

Write down everything you eat, beginning with the first thing you have for breakfast. Be sure to include very detailed information such as how the food was prepared, how much you ate, and the brand names.

Time Eaten	Foods Eaten	Preparation Methods	Amount (cup, 1/2 cup, piece, etc)

Please continue on the back of page if necessary.

APPENDIX F

Accelerometer Recording Sheet



PLEASE COMPLETE BY: _____

ACTIVITY MONITOR INSTRUCTIONS

- Attach monitor to your daughter's waist
- Wear for **THREE (3) days: 2 weekdays and 1 weekend day**
- Monitor should be worn at all times, from wake-up time, until bedtime
EXCEPT: during baths and/or swimming
- Please complete the 3-day period by the date at the top of the page
- Record days, dates and time the monitor was worn in the spaces below:

☆ ~***AND RETURN THIS SHEET WITH THE MONITOR***~ ☆

DAY ONE: **day:** **date:** **time on:** **time off:**

DAY TWO: **day:** **date:** **time on:** **time off:**

DAY THREE: **day:** **date:** **time on:** **time off:**

(DAY FOUR if applicable): **day:** **date:** **time on:** **time off:**

CAUTIONS

- *Never get the monitor wet*
- *Please check clothing before washing to avoid laundering*
- *Tape is placed around the monitor case so it cannot be opened*

If you have questions about the monitor, please call:

Dr. Richard Lewis- (706) 542-4901

Dr. Pat O'Connor- (706) 542-4382

Emma Laing- (706) 542-4918

THANK YOU FOR YOUR PARTICIPATION IN OUR STUDY!

APPENDIX G

Demographic Questionnaire

Subject ID#: _____

Interviewer: _____

Date of Interview: _____

Demographic Data:

I am going to ask you some questions about your age, family, and education. Your mother or father can help you answer.

1. What is your date of birth? Month _____ Day _____ Year _____
2. What is your age? Years _____ Months _____
3. Gender: (Circle One) Female Male
4. What is your race? (Circle One) Caucasian
Black
Asian
Hispanic
American Indian
Other _____
5. Do you live with your parents? (Circle One) YES NO
5a. If no, with whom do you live? _____
6. Do you have any brothers or sisters? (Circle One) YES NO
6a. If yes, list ages of: _____ Years (Brother) _____ Years (Sister)
_____ Years (Brother) _____ Years (Sister)
_____ Years (Brother) _____ Years (Sister)
6b. If yes, do they participate in gymnastics or others sports?
(Circle One) YES NO
6c. If yes, list the sport and gender of sibling.
Sport _____ (Brother or Sister)
Sport _____ (Brother or Sister)
Sport _____ (Brother or Sister)
Sport _____ (Brother or Sister)
7. Do you have a twin sister? (Circle One) YES NO
8. At what age did you start gymnastics? _____ Years _____ Months
9. Was your mother a gymnast? (Circle One) YES NO

Subject ID#: _____

Interviewer: _____

Date of Interview: _____

10. What is your parents income? (Circle One)
- Less than \$9,999
 - \$10,000 - \$19,999
 - \$20,000 - \$29,999
 - \$30,000 - \$39,999
 - \$40,000 - \$49,999
 - \$50,000 - \$59,999
 - \$60,000 - \$69,999
 - \$70,000 - \$79,999
 - \$80,000 - \$89,999
 - \$90,000 - \$99,999
 - More than \$100,000

11. What grade are you in school? (Circle One) Kindergarten 1st 2nd 3rd

12. What is your mother's occupation? _____

13. What is your father's occupation? _____

Subject ID#: _____

Interviewer: _____

Date of Interview: _____

Health Data

I am going to ask you to respond to a few questions about your health. I am the only one that will know how your responses to these questions, so please be honest with your answers.

1. How much do you weigh? _____pounds (Actual scale weight _____ lbs.)

2. How tall are you? _____feet _____inches

3. BMI=_____ (Interviewer to complete later)

4. Have you gained or lost any weight (≥ 10 pounds) in the past 3 months?

(Circle One) YES NO

4a. If yes, how much? +_____pounds OR -_____pounds

5. Have you had any height changes in the past 3 months?

(Circle One) YES NO

5a. If yes, how much? _____feet _____inches

6. How much would you like to weigh? _____pounds

7. How tall would you like to be? _____feet _____inches

8. How would you rate your present health? (Circle One)

Poor

Fair

Good

Excellent

9. Have you started your menstrual cycles? (Circle One) YES NO

If so, what date?

10. Do you have any diseases or illnesses? (Circle One) YES NO

10a. If yes, what diseases?

11. Are you taking any medications either prescribed by a doctor or over-the-counter

(self-prescribed)? (Circle One) YES NO

11a. If yes, what medications?

Amount per day _____

Amount per day _____

Those were some difficult questions to answer because the questions were so private. I want to assure you again that I am the only person who knows how you answered these questions. Thank you for being so honest with your answers.

Subject ID#: _____

Interviewer: _____

Date of Interview: _____

Nutrition Data:

These next questions are about your eating habits. Try to think about how you eat.

1. Do you eat three meals per day? (Circle One) YES NO
1a. If no, why not? _____
2. Do you eat snacks during the day? (Circle One) YES NO
2a. If yes, how many snacks per day do you eat? _____ snacks per day
3. Are you following a special kind of diet? (Circle One) YES NO
3a. If yes, what kind of diet? _____
4. Do you take any vitamin or mineral supplements or any “nutrition pills”?
(Circle One) YES NO
4a. If yes, what kind? _____ Amount per day _____
_____ Amount per day _____
_____ Amount per day _____
5. Have you ever been on a diet to lose weight? (Circle One) YES NO
5a. If yes, what kind of a diet was it? _____
5b. How old were you when you were on this diet?
_____ years _____ months
6. Have you ever eaten a large amount of food and then vomited to get rid of the food?
(Circle One) YES NO
6a. If yes, how old were you? _____ years _____ months
_____ years _____ months
7. Have you ever starved yourself for more than three days?
(Circle One) YES NO
7a. If yes, how old were you? _____ years _____ months
_____ years _____ months

Thank you for answering all of those questions. You did really well, and I appreciate your being so truthful with your answers. Next, I am going to ask you about your physical activity during the past 7 days. Try to think back on last week and the activities that you may have done.

Subject ID#: _____

Interviewer: _____

Date of Interview: _____

Bone Health Data:

The next questions have to do with your bones and your family's bones.

1. Does anyone in your family (including your parent's, grandparents, aunts, uncles, cousins) have osteoporosis or "humpback"? (Circle One) YES NO
1a. If yes, who is it? _____
2. Has anyone in your family (including your parents, grandparents, aunts, uncles, cousins) had a hip or wrist fracture? (Circle One) YES NO
2a. If yes, who is it? _____
3. Have you ever had a bone fracture or broken bone? (Circle One) YES NO
3a. If yes, what bone(s)? _____
3b. If yes, how old were you? _____ years _____ months
4. Have you ever been told by a doctor that you have bone disease?
(Circle One) YES NO
4a. If yes, what disease? _____
4b. If yes, how old were you? _____ years _____ months

Subject ID#: _____

Interviewer: _____

Date of Interview: _____

Physical Activity

The next questions that I will ask you are about your physical activity such as P.E., recess, and exercise. There are no right or wrong answers, so please answer these questions the best that you can.

1. How would you rate your physical activity level? (Circle One)
Inactive
Below average
Average
Above average
Very high
2. Do you have any health problems that limit your activity?
(Circle One) YES NO
 - 2a. If yes, what health problem? _____
3. Do you exercise regularly (not including P.E. class)? (Circle One) YES NO
 - 3a. If yes, how often? _____ hours per day/week/month (Circle One)
4. Do you participate in P.E. at school? (Circle One) YES NO
 - 4a. If yes, how often? _____ hours per day/week/month (Circle One)
5. Do you play games during recess? (Circle One) YES NO
 - 5a. If yes, what games or activities do you play? _____

 - 5b. If yes, how many hours per day do you play during recess?
_____ hours per day
6. Do you play games after school? (Circle One) YES NO
 - 6a. If yes, what games or activities do you play? _____

 - 6b. If yes, how many hours per day do you play after school?
_____ hours per day

APPENDIX H

Physical Activity Questionnaire

APPENDIX
Children's questionnaire

Abridged questions and all possible responses:

1. Frequency of physical education classes
0 1 2 3 4 5 times per week _____ other
2. Length of physical education classes.
0 <20 21-25 26-30 31-35 36-40 41-45 46-50 60+ minutes
3. Proportion of class time spent in intense activities (activities that make you breathe hard).
None 1/4 1/2 3/4 all
4. Television watched.

School nights none 1/2-1 1.5-2 2.5-3 3.5-4 4.5-5 5.5+
Nonschool nights none 1/2-1 1.5-2 2.5-3 3.5-4 4.5-5 5.5+
5. Hours per week spent in each listed activity.

	0	1-2	3-4	5-6	7-8	9-10	11+
Cycling	_____	_____	_____	_____	_____	_____	_____
Swimming	_____	_____	_____	_____	_____	_____	_____
Running	_____	_____	_____	_____	_____	_____	_____
Running games	_____	_____	_____	_____	_____	_____	_____
Weight lifting	_____	_____	_____	_____	_____	_____	_____
Aerobic dance	_____	_____	_____	_____	_____	_____	_____
Walking	_____	_____	_____	_____	_____	_____	_____
Baseball	_____	_____	_____	_____	_____	_____	_____
Basketball	_____	_____	_____	_____	_____	_____	_____
Soccer	_____	_____	_____	_____	_____	_____	_____
Tennis	_____	_____	_____	_____	_____	_____	_____
6. Participation in team sports. All yes/no
Baseball/softball Football Basketball
Gymnastics Swimming Track
Soccer Cross-country Tennis
Volleyball Golf Wrestling
7. Open-ended list. Four most frequent activities. Time spent in each per week.

Mother's questionnaire

1. Time spent in vigorous activity on an average day.
None about 1 2 3 4 5+ hours
2. Level of child's activity compared to other children the same age.
Much less less same more much more
3. Television watched by the child.

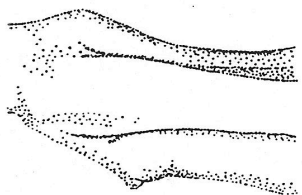
School nights none 1/2-1 1.5-2 2.5-3 3.5-4 4.5-5 5.5+
Nonschool nights none 1/2-1 1.5-2 2.5-3 3.5-4 4.5-5 5.5+
4. Open-ended list. Four most frequent activities for each child. Time spent in each per week.

APPENDIX I

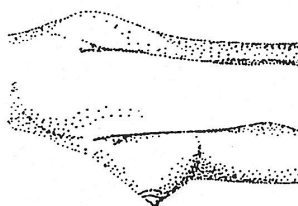
Tanner Stages of Sexual Maturation



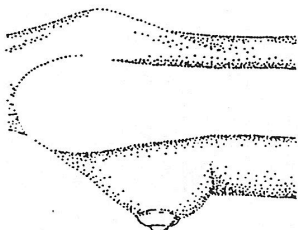
1 Prepubertal



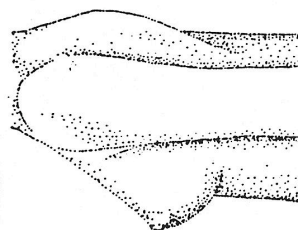
2 Breast Bud



3 Breast Elevation



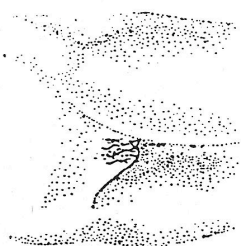
4 Areolar Mound



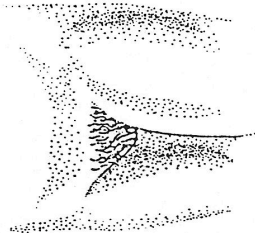
5 Adult Contour



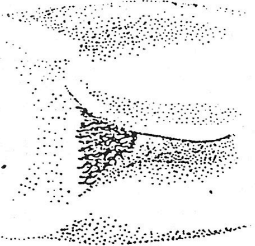
1 Prepubertal



2 Presexual hair



3 Sexual hair



4 Mid-escutcheon



5 Female escutcheon

Tanner Staging	Breast	Pubic Hair
Stage 1 (prepubertal)	Elevation of papilla only	No pubic hair
Stage 2	Elevation of breast and papilla as small mound, areola diameter enlarged. Median age: 9.8 years	Sparse, long, downy hair chiefly along labia majora. Median age: 10.5 years
Stage 3	Further enlargement without separation of breast and areola. Median age: 11.2 years	Dark, coarse, curled hair sparsely spread over mons. Median age: 11.4 years
Stage 4	Secondary mound of areola and papilla above the breast. Median age: 12.1 years	Adult-type hair, abundant but limited to the mons. Median age: 12.0 years
Stage 5	Recession of areola to contour of breast. Median age: 14.6 years	Adult type spread in quantity and distribution. Median age: 13.7 years

APPENDIX J

Anthropometric Data Recording Sheet

**BONE, GROWTH AND DIETARY INTAKES
IN 4-8 YEAR OLD GIRLS**

ANTHROPOMETRIC DATA SHEET

ID NUMBER: _____ time _____

HEIGHT _____ (TO NEAREST 1/4 INCH)

WEIGHT _____ (TO NEAREST 1/4 POUND)

LEG LENGTH _____ (TO NEAREST 1/4 INCH)

SITTING HEIGHT _____ (TO NEAREST 1/4 INCH)

MOM'S HEIGHT _____ (TO NEAREST 1/4 INCH)
SELF-REPORT? YES NO

DAD'S HEIGHT _____ (TO NEAREST 1/4 INCH)
SELF-REPORT? YES NO

LENGTH OF RADIUS IN CENTIMETERS _____ R or L? _____

NUMBER OF BLOCKS USED FOR SPINE SCAN _____

Hip Circumference: _____ Belly Circumference: _____ Waist Circumference: _____

BIRTHDATE _____

TO BE COMPLETED BY INVESTIGATOR:

PREDICTED HEIGHT _____

% PREDICTED HEIGHT _____

GROWTH VELOCITY

HEIGHT _____

LEG LENGTH _____

SITTING HEIGHT _____

% HEIGHT FOR AGE _____

% WEIGHT FOR AGE _____

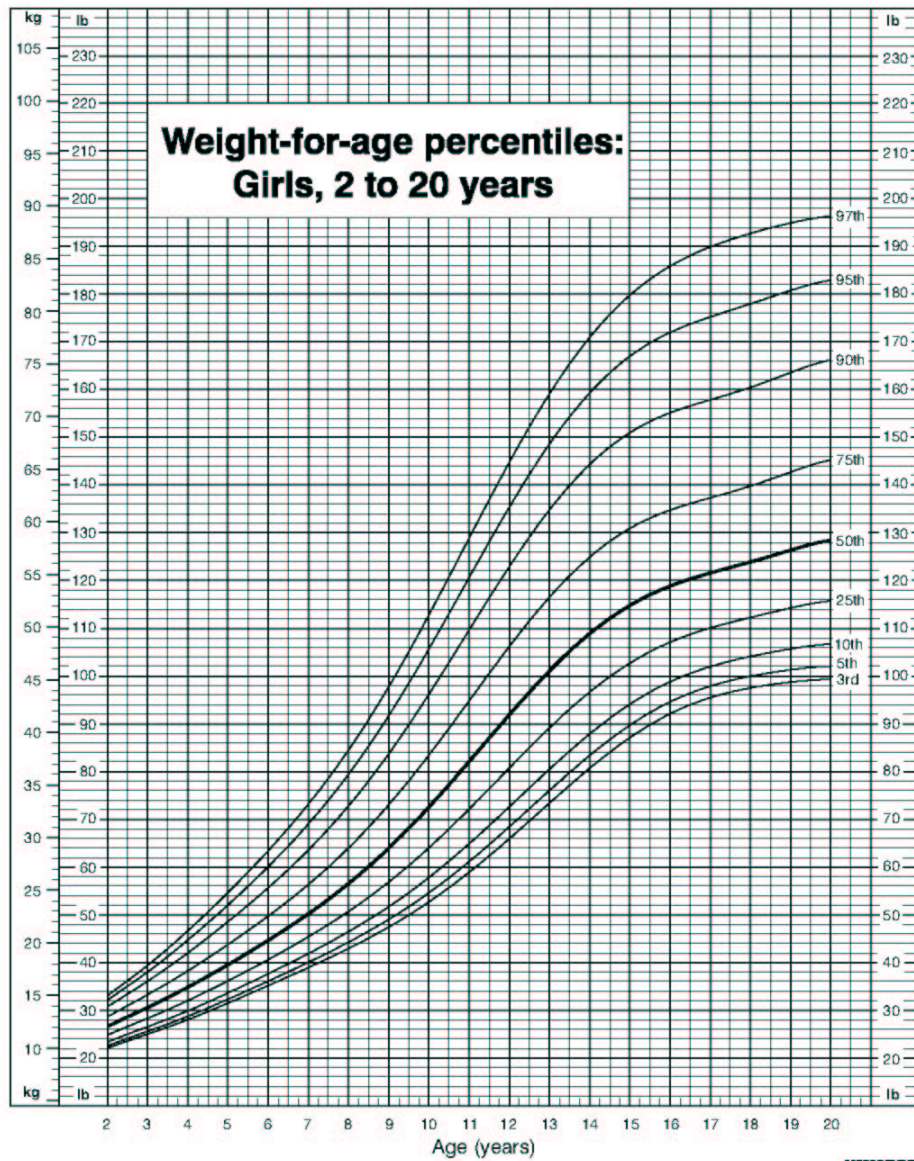
% WEIGHT FOR HEIGHT _____

BMI _____

APPENDIX K

Growth and Body Mass Index Charts

CDC Growth Charts: United States



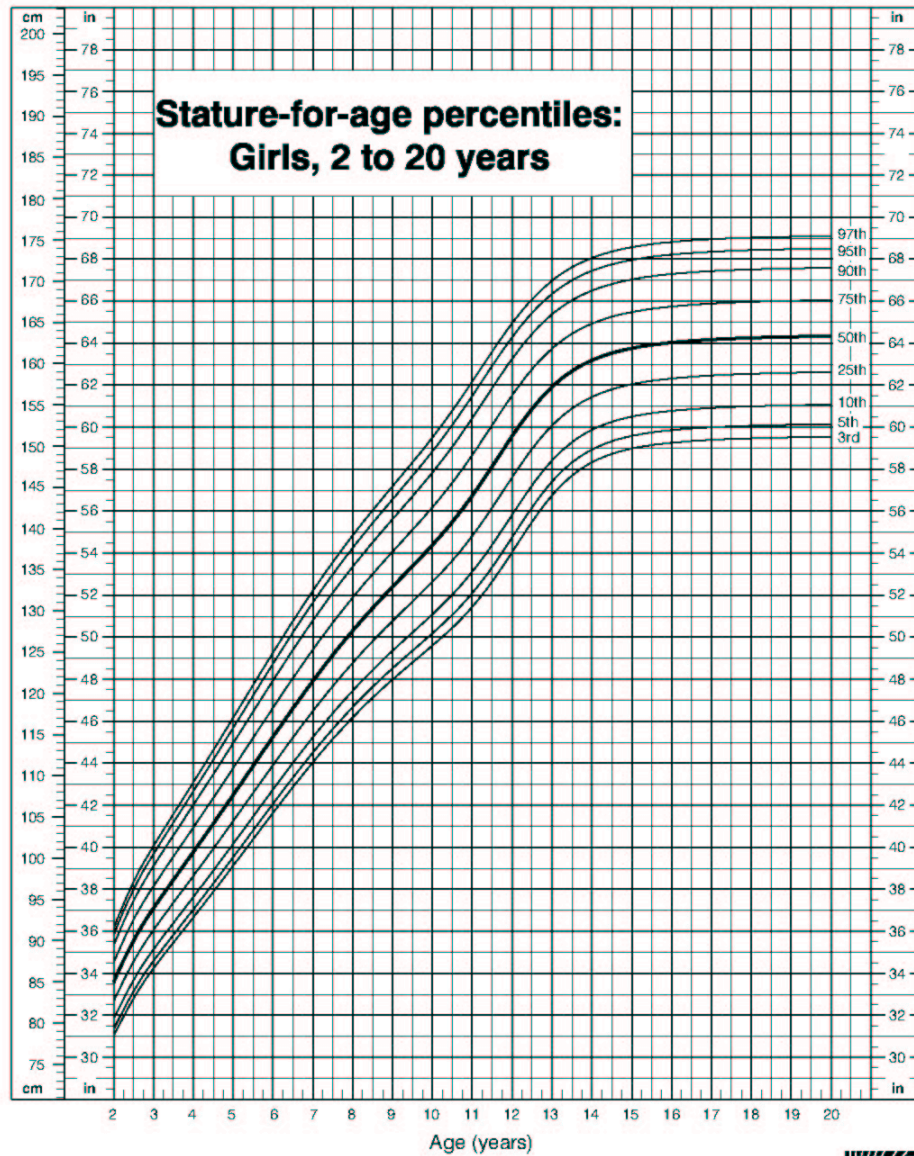
Published May 30, 2000.

SOURCE: Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000).



SAFER • HEALTHIER • PEOPLE™

CDC Growth Charts: United States



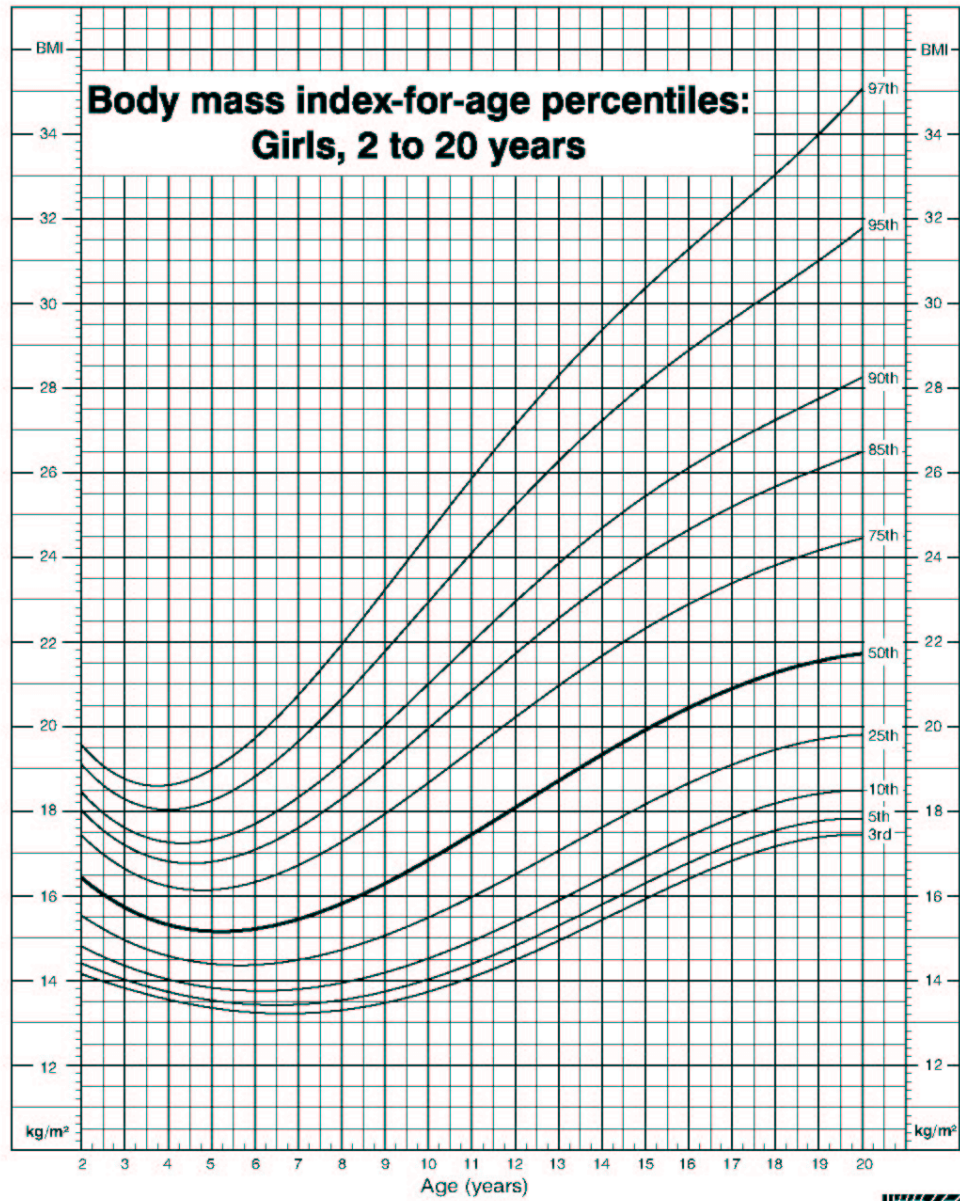
Published May 30, 2000.

SOURCE: Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000).



SAFER • HEALTHIER • PEOPLE™

CDC Growth Charts: United States



Published May 30, 2000.
SOURCE: Developed by the National Center for Health Statistics in collaboration with
the National Center for Chronic Disease Prevention and Health Promotion (2000).



APPENDIX L

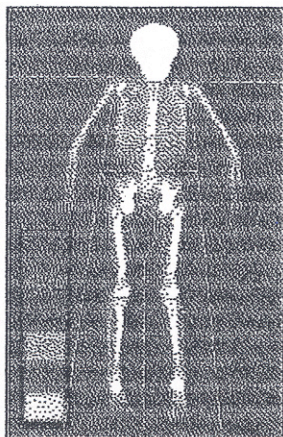
Dual Energy X-Ray Absorptiometry Scans:

Total Body
Lumbar Spine
Total Proximal Femur
Forearm

CLINICAL & SPORTS NUTRITION LAB~UGA

J06260001 Mon Jun 26 07:33 2000

Name: vygym
 Comment: baseline
 I.D.: Sex: F
 S.S.#: - - Ethnic: C
 ZIP Code: Height: 3' 11"
 Scan Code: Weight: 52
 BirthDate: 07/12/92 Age: 7
 Physician:
 Image not for diagnostic use



Jun 29 16:09 2000 [336 x 1071]
 HOLOGIC QDR-1000/W (S/N 1101 P)
 Experimental Pediatric WB V5.73

TOTAL BMC and BMD CV is < 1.0%

C.F.	1.010	1.104	1.000
Region	Area (cm ²)	BMC (grams)	BMD (gms/cm ²)
L Arm	125.39	48.65	0.388
R Arm	116.92	46.59	0.398
L Ribs	62.49	25.74	0.412
R Ribs	53.62	21.40	0.399
T Spine	53.22	26.65	0.501
L Spine	31.85	19.36	0.608
Pelvis	136.68	94.92	0.694
L Leg	220.94	142.48	0.645
R Leg	259.24	167.02	0.644
SubTot	1060.36	592.81	0.559
Head	171.75	210.35	1.225
TOTAL	1232.11	803.16	0.652

HOLOGIC

CLINICAL & SPORTS NUTRITION LAB~UGA

J06260001 Mon Jun 26 07:33 2000

Name: vygym
 Comment: baseline
 I.D.: Sex: F
 S.S.#: - - Ethnic: C
 ZIP Code: Height: 3' 11"
 Scan Code: Weight: 52
 BirthDate: 07/12/92 Age: 7
 Physician:

HOLOGIC QDR-1000/W (S/N 1101 P)
 Experimental Pediatric WB V5.73
 Jun 29 16:09 2000

TBAR414
 F.S. 68.00% 0(10.00)%

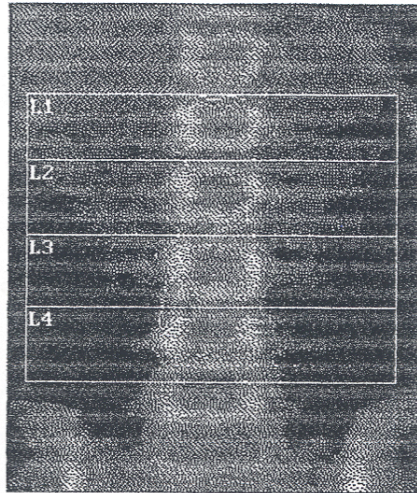
Region	BMC (grams)	Fat (grams)	Lean (grams)	Lean+BMC (grams)	Total (grams)	% Fat (%)
L Arm	48.7	465.8	624.6	673.2	1139.0	40.9
R Arm	46.6	537.7	563.8	610.4	1148.1	46.8
Trunk	188.1	2406.0	7216.3	7404.4	9810.4	24.5
L Leg	142.5	2013.4	1631.6	1774.1	3787.5	53.2
R Leg	167.0	2083.5	1470.4	1637.4	3720.9	56.0
SubTot	592.8	7506.4	11506.8	12099.6	19606.0	38.3
Head	210.4	458.4	2125.8	2336.1	2794.6	16.4
TOTAL	803.2	7964.8	13632.6	14435.7	22400.5	35.6

*assumes 17.0% brain fat
 LBM 73.2% water

HOLOGIC

CLINICAL & SPORTS NUTRITION LAB~UGA

k = 1.242 d0 = 125.1(1.000H)



Jun 29 16:10 2000 [118 x 93]
HOLOGIC QDR-1000/W (S/N 1101 P)
Low Density Spine V4.76P

J06260002 Mon Jun 26 07:46 2000

Name: vygym
Comment: baseline
I.D.: Sex: F
S.S.#: - - Ethnic: C
ZIP Code: Height: 3'11"
Scan Code: Weight: 52
BirthDate: 07/12/92 Age: 7
Physician:
Image not for diagnostic use

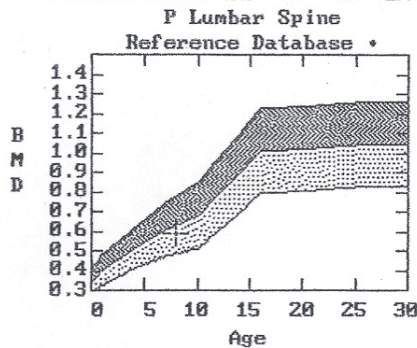
TOTAL BMD CV FOR L1 - L4 1.0%

C.F. 1.010 1.104 1.000

Region	Area (cm ²)	BMC (grams)	BMD (gms/cm ²)
L1	5.73	3.05	0.532
L2	7.13	3.94	0.553
L3	7.58	4.66	0.615
L4	8.81	5.40	0.613
TOTAL	29.24	17.05	0.583

HOLOGIC

CLINICAL & SPORTS NUTRITION LAB~UGA



BMD(L1-L4) = 0.583 g/cm²

Region	BMD	T(30.0)	Z
L1	0.532	-3.57 58%	
L2	0.553	-4.32 54%	
L3	0.615	-4.26 57%	
L4	0.613	-4.57 55%	
L1-L4	0.583	-4.22 56%	-0.62 93%

♦ Age and sex matched

T = peak BMD matched

Z = age matched

TK 11/04/91

J06260002 Mon Jun 26 07:46 2000

Name: vygym
Comment: baseline
I.D.: Sex: F
S.S.#: - - Ethnic: C
ZIP Code: Height: 3'11"
Scan Code: Weight: 52
BirthDate: 07/12/92 Age: 7
Physician:

HOLOGIC

CLINICAL & SPORTS NUTRITION LAB~UGA

k = 1.252 d0 = 126.3(1.000H)



Jun 29 16:13 2000 [64 x 83]
HOLOGIC QDR-1000/W (S/N 1101 P)
Left Hip V4.76P

J06260003 Mon Jun 26 07:54 2000

Name: vygym
Comment: baseline
I.D.: Sex: F
S.S.#: - - Ethnic: C
ZIP Code: Height: 3' 11"
Scan Code: Weight: 52
BirthDate: 07/12/92 Age: 7
Physician:

Image not for diagnostic use

TOTAL BMD CV 1.0%

C.F. 1.010 1.104 1.000

Region	Area (cm ²)	BMC (grams)	BMD (gms/cm ²)
--------	-------------------------	-------------	----------------------------

Neck	2.83	1.55	0.548
------	------	------	-------

Troch	4.51	2.30	0.510
-------	------	------	-------

Inter	9.65	6.60	0.684
-------	------	------	-------

TOTAL	16.98	10.45	0.615
-------	-------	-------	-------

Ward's	1.24	0.67	0.541
--------	------	------	-------

Midline (70, 92)-(108, 62)

Neck -51 x 12 at I 25, 151

Troch 3 x 25 at I 0, 01

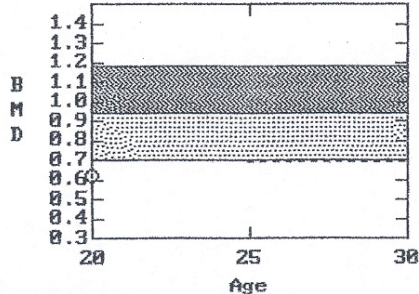
Ward's -11 x 11 at I 5, 51



CLINICAL & SPORTS NUTRITION LAB~UGA

P Left Hip

Reference Database *



BMD(Total[LL]) = 0.615 g/cm²

Region	BMD	T	Z
Neck	0.548	-2.71 65% (25.0)	
Troch	0.510	-1.91 73% (25.0)	
Inter	0.684	-2.69 62% (35.0)	
TOTAL	0.615	-2.68 65% (25.0)	
Ward's	0.541	-1.65 74% (25.0)	

♦ Age and sex matched

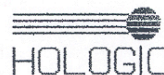
T = peak BMD matched

Z = age matched

NHA 02/01/97

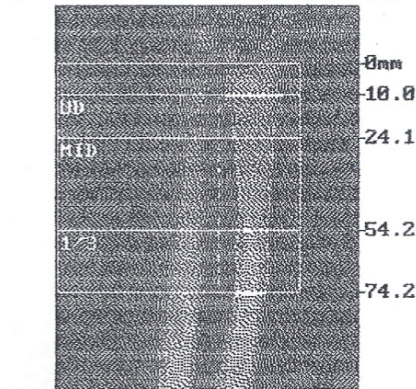
J06260003 Mon Jun 26 07:54 2000

Name: vygym
Comment: baseline
I.D.: Sex: F
S.S.#: - - Ethnic: C
ZIP Code: Height: 3' 11"
Scan Code: Weight: 52
BirthDate: 07/12/92 Age: 7
Physician:



CLINICAL & SPORTS NUTRITION LAB~UGA

k = 1.411 d0 = 155.3(1.000)[4]



Jun 29 16:14 2000 [156 x 38]
HOLOGIC QDR-1000/W (S/N 1101 P)
Left Forearm V5.73Q

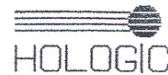
J06260004 Mon Jun 26 08:00 2000

Name: vygym
Comment: baseline
I.D.: Sex: F
S.S.#: - - Ethnic: C
ZIP Code: Height: 3'11"
Scan Code: Weight: 52
BirthDate: 07/12/92 Age: 7
Physician:
Forearm Length: 19.0 cm
Image not for diagnostic use

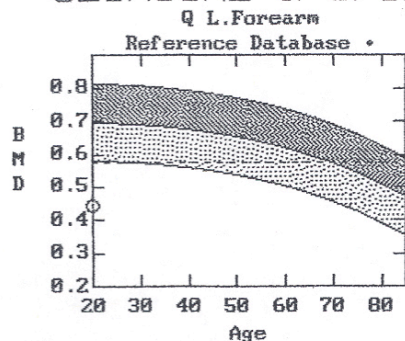
TOTAL BMD CV IS LESS THAN 1.0%

C.F. 1.010 1.104 1.000

RADIUS	Area (cm2)	BMC (grams)	BMD (gms/cm2)
UD	2.19	0.66	0.302
MID	2.69	0.97	0.361
1/3	2.02	0.89	0.439
TOTAL	6.90	2.52	0.365



CLINICAL & SPORTS NUTRITION LAB~UGA



BMD(Radius[L] 1/3) = 0.439 g/cm²

Region	BMD	T	Z
1/3	0.439	-4.25 63% (20.0)	
MID	0.361	-4.49 59% (20.0)	
UD	0.302	-2.43 68% (20.0)	
TOTAL	0.365	-3.96 63% (20.0)	

* Age and sex matched

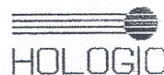
T = peak BMD matched

Z = age matched

PS 10/25/91

J06260004 Mon Jun 26 08:00 2000

Name: vygym
Comment: baseline
I.D.: Sex: F
S.S.#: - - Ethnic: C
ZIP Code: Height: 3'11"
Scan Code: Weight: 52
BirthDate: 07/12/92 Age: 7
Physician:



APPENDIX M

The Fels Method for Skeletal Age Assessment

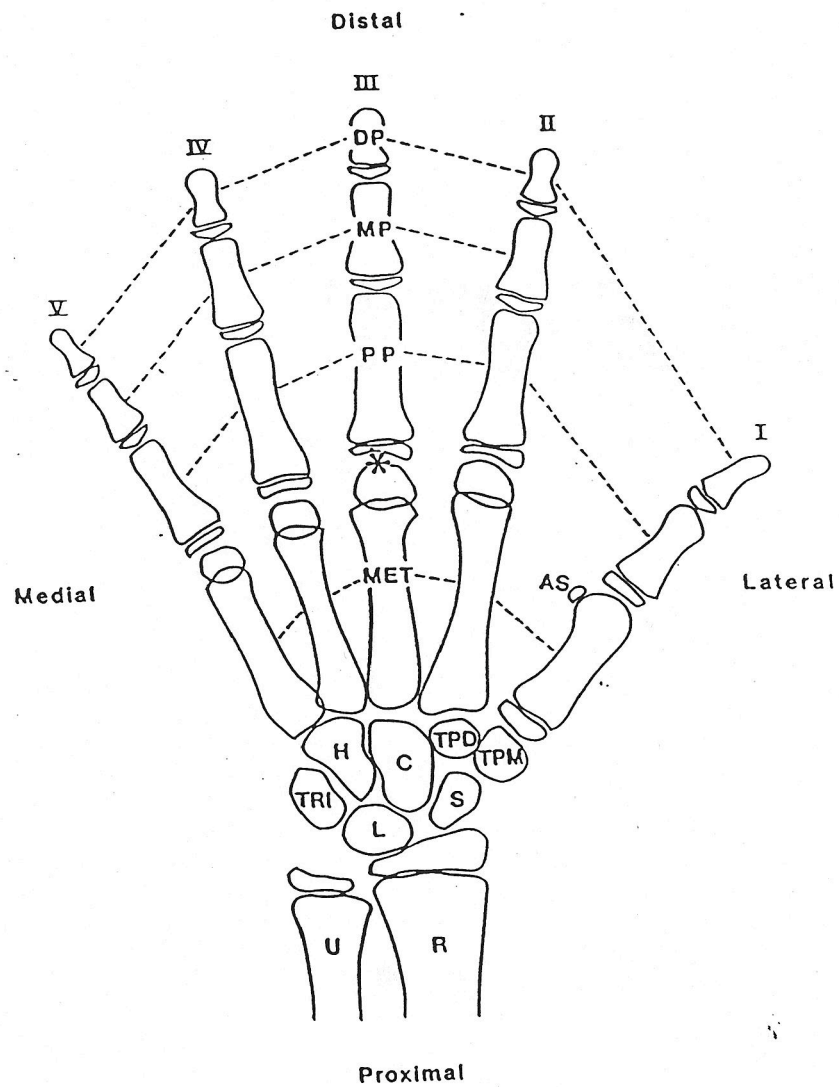
THE RECORDING FORM
FELS HAND-WRIST SKELETAL AGE

Name _____		Assessor No. _____		Skeletal Age _____	
Birthdate _____		Sex _____		Assessment Date _____	
Chron. Age _____		Race _____		X-Ray Date _____	
				Film Condition _____	

RADIUS	TRIQUETRAL	TPD-6 _____
R-1 _____	TRI-1 _____	TPD-7 _____
R-2 _____	TRI-2 _____	ADDUCTOR
(EW) _____ m m	TRI-3 _____	SESAMOID
(MW) _____ m m	TRI-4 _____	AS-1 _____
R-3 _____	PISIFORM	METACARPAL I
R-4 _____	P-1 _____	MET I-1 _____
R-5 _____	LUNATE	MET I-2 _____
R-6 _____	L-1 _____	(EW) _____ m m
R-7 _____	L-2 _____	(MW) _____ m m
R-8 _____		
ULNA	SCAPHOID	MET I-3 _____
U-1 _____	S-1 _____	MET I-4 _____
U-2 _____	S-2 _____	MET I-5 _____
(EW) _____ m m	S-3 _____	MET I-6 _____
(MW) _____ m m	TRAPEZIUM	MET I-7 _____
U-3 _____	TPM-1 _____	METACARPAL III
CAPITATE	TPM-2 _____	MET III-1 _____
C-1 _____	TPM-3 _____	MET III-2 _____
C-2 _____	TPM-4 _____	(EW) _____ m m
C-3 _____	TPM-5 _____	(MW) _____ m m
C-4 _____	TRAPEZOID	MET III-3 _____
HAMATE	TPD-1 _____	MET III-4 _____
H-1 _____	TPD-2 _____	MET III-5 _____
H-2 _____	TPD-3 _____	METACARPAL V
H-3 _____	TPD-4 _____	MET V-1 _____
H-4 _____	TPD-5 _____	MET V-2 _____

314 Assessing the Skeletal Maturity of the Hand-Wrist: FELS Method

(EW) _____ m m	PP III-5 _____	(MW) _____ m m
(MW) _____ m m	PP III-6 _____	MP V-3 _____
MET V-3 _____	PROXIMAL	MP V-4 _____
MET V-4 _____	PHALANX V	MP V-5 _____
MET V-5 _____	PP V-1 _____	DISTAL PHALANX I
MET V-6 _____	PP V-2 _____	DP I-2 _____
PROXIMAL	(EW) _____ m m	(EW) _____ m m
PHALANX I	(MW) _____ m m	(MW) _____ m m
PP I-1 _____	PP V-3 _____	DP I-4 _____
PP I-2 _____	PP V-4 _____	DISTAL
(EW) _____ m m	PP V-5 _____	PHALANX III
(MW) _____ m m	MIDDLE	DP III-1 _____
PP I-3 _____	PHALANX III	DP III-2 _____
PP I-4 _____	MP III-1 _____	(EW) _____ m m
PP I-5 _____	MP III-2 _____	(MW) _____ m m
PP I-6 _____	(EW) _____ m m	DP III-3 _____
PP I-7 _____	(MW) _____ m m	DP III-4 _____
PROXIMAL	MP III-3 _____	DISTAL
PHALANX III	MP III-4 _____	PHALANX V
PP III-1 _____	MP III-5 _____	DP V-1 _____
PP III-2 _____	MIDDLE	DP V-2 _____
(EW) _____ m m	PHALANX V	(EW) _____ m m
(MW) _____ m m	MP V-1 _____	(MW) _____ m m
PP III-3 _____	MP V-2 _____	DP V-3 _____
PP III-4 _____	(EW) _____ m m	DP V-4 _____



POSITIONING

HAND:

Place the forearm, palm and fingers flat on the table. A clear plastic paddle can be used to keep the fingers extended in young children.

Separate the fingers slightly and align the third finger with the forearm.

Direct the central ray at the distal end of Metacarpal III with a tube-to-film distance of 36".